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SIXTH FRAMEWORK PROGRAMME



# Optimised Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information

## Optimised Strategies for the Risk Assessment of Chemicals

According to REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), the European legislation on chemicals and their safe use, all industrial chemicals produced or imported in quantities above 1 t/y have to be evaluated regarding their toxicological and ecotoxicological effects. Considering the currently used testing schemes, this procedure is expected to result in a significant increase in animal tests. However, REACH also aims at reducing animal testing where possible.

OSIRIS is developing Integrated Testing Strategies (ITS) considering both non-test and test information and thus combining different approaches for the hazard and risk evaluation of chemicals. ITS shift risk assessment from a "box-ticking" approach with extensive animal testing to a more efficient, context-specific and substance-tailored approach.

The complementary alternative approaches considered include:

- Chemical and biological read-across
- Qualitative/quantitative structure-activity relationships (QSAR)
- In vitro testing
- In vivo information (existing)
- Chemoassays
- -omics
- Thresholds of toxicological concern (TTC)
- Exposure analysis and exposure-based waiving.



Integrated Testing Strategies are developed for the human health and environmental endpoints:

- Skin sensitisation
- Repeated dose toxicity
- Mutagenicity & carcinogenicity
- Bioconcentration factor
- Aquatic toxicity.



## OSIRIS Partners

OSIRIS has 31 Partners from 14 European countries.  
24 Research institutes / universities  
5 Small and medium-sized enterprises  
2 Manufacturers of chemicals and chemical products

## The OSIRIS Integrated Testing Strategies

The underlying principle of Integrated Testing Strategies (ITS) is to take advantage of existing information, to group information about similar substances, and to integrate exposure considerations in the decision making. The different information is weighted and the respective uncertainties taken into account in a Weight of Evidence approach.

Thus an ITS combines all available testing and non-testing data and concludes whether or not additional data is needed. In case of data gaps, the ITS proposes the most appropriate method to acquire the missing information. Ideally, with regard to the 3R principle of replacement, reduction and refinement of animal testing, non-testing methods such as *in vitro* assays and QSAR (qualitative or quantitative structure-activity relationship) methods are preferred for this purpose.

The ITS use endpoint-specific testing and non-testing methods and weight their contribution:

- Step 1: Gather all substance-specific information (testing and non-testing data)
- Step 2: Add weight to type of information using statistical methods and/or expert knowledge
- Step 3: Conclude whether gathered information and/or performed *in vitro* testing are sufficient for classification & labelling (C&L) / risk assessment

If data are not sufficient for C&L or risk assessment – data gap is identified:

- Step 4: Gather information on structurally related

chemicals to do read-across or category approach/  
perform *in vitro* testing if technically possible and relevant for respective endpoint

- Step 5: Is exposure-based waiving (EBW) an option? Are thresholds of toxicological concern (TTC) an option? Does the compound belong to the applicability domain of TTC?
- Step 6: Propose animal testing as last resort.

Some specific issues of the ITS for the different endpoints are discussed in the following.

## ITS Skin Sensitisation

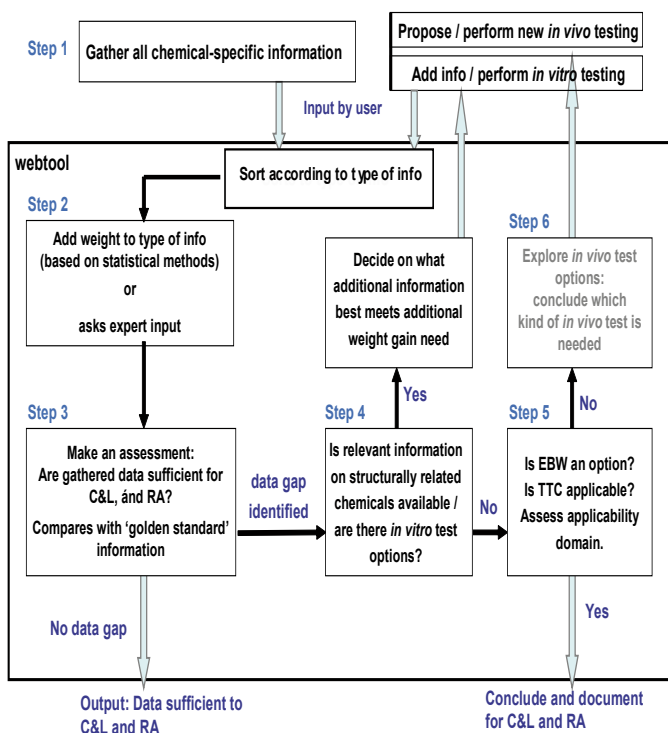
In experimental testing often a minimum concentration causing sensitisation above a specific threshold can be observed. This concentration determines the skin sensitisation potential of a substance. For REACH, information on the skin sensitisation potential is not required, only information whether a substance in any concentration is capable of causing at least a 3 fold increase of the stimulation index in a Local Lymph Node Assay (LLNA). Therefore skin sensitisation is treated as a categorical (yes/no) endpoint in the OSIRIS ITS.

For this endpoint, a multitude of alternative methods is available: older test types, *in vitro* assays and a number of QSAR models predicting the ability to cause skin sensitisation. Bayesian decision theory is applied to calculate the probability for each single alternative and/or combination of these alternative methods that a test/model outcome is correct. All methods integrated in the ITS have to be characterised quantitatively in terms of sensitivity and specificity of predicting the required test result, i.e. the LLNA test. This probability (as a percentage) can subsequently be compared to the probability that the LLNA test is giving the “true” result after only one test. Thus an objective, transparent, but also strictly statistical threshold is generated which determines whether the available tests/models deliver sufficient “Weight of Evidence” to fulfil REACH registration purposes. The quantification of the “weight” of each method also creates the possibility to define the most optimal “next step” in testing.

The ITS considers available OSIRIS and public data:

- Human: Patch Test data (HPT)
- In vivo: Guinea Pig Maximisation Test (GPMT) incl. Buehler assay, Murine Local Lymph Node Assay
- In vitro: Human Cell line activation test
- In silico: (QSAR) models, OASIS TIMES-S (Tissue Metabolism Simulator-Skin sensitisation), MultiCASE model (Danish EPA QSAR database), Derek for Windows (Lhasa Ltd), SMARTs rules (LJMU), Accelrys TOPKAT.

Any other test or model predicting the endpoint skin sensitisation, which can be characterised in terms of its sensitivity and specificity in predicting the LLNA test, can be further included in this ITS.



General scheme of the OSIRIS Integrated Testing Strategies for human health endpoints.

## ITS Repeated Dose Toxicity

Repeated dose toxicity is a complex endpoint for which mode and mechanism of action are highly substance-dependent and normally not known. Unspecific cytotoxicity, receptor-induced toxicity or a combination of both may play a role. Moreover, complex biological processes as distribution, metabolism and elimination influence the overall toxicity. So far single or combined in vitro assays are not able to mimic all relevant in vivo processes and are thus not able to substitute parts or whole in vivo repeated dose studies. Therefore in vitro assays have neither been considered in step 2 nor step 6 of the repeated dose toxicity ITS. Furthermore, only few QSAR methods for repeated dose toxicity have been described so far.

The ITS focuses on step 2, where the reliability/relevance of the available testing data is assessed. In many cases, toxicity data of existing chemicals are available from "old" studies conducted before the publication of OECD guidelines. The results of these non-guideline studies are now considered as not reliable or only with restrictions. However, also "old" studies may be valid. This validity of the non-guideline study depends on the scope of examination and the overall quality of the study. Therefore a major focus of the repeated dose toxicity ITS – before concluding on the data gap and continuing with the following steps – is to assess data validity and specifically to which extent and under which conditions non-guideline studies are valid to be included in the risk assessment.

## ITS Mutagenicity & Carcinogenicity

The outcome of mutagenicity testing influences the subsequent concern and testing strategy for carcinogenic properties. Positive results in mutagenicity assays raise concern for (genotoxic) carcinogenicity and generally lead to precautionary labelling, whereas non-genotoxic carcinogens cannot be identified using mutagenicity testing. Genotoxic carcinogens can further be subdivided in substances that directly react with DNA molecules, and substances that induce genotoxicity indirectly. For a comprehensive coverage of the potential mutagenic properties of a substance, information on its ability to induce gene mutations, structural and numerical chromosomal aberrations is required.

The testing strategy of the ITS for [mutagenicity](#) according to the REACH requirements consists of a number of well accepted in vitro tests, each testing a different aspect of mutagenicity. They should be regarded as separate golden standards. In vivo tests have the function to select true in vitro positives, i.e. (existent) in vivo test data overrule in vitro test results and can therefore also substitute the corresponding in vitro test. Consequently, the strategy changes when information is available, the next step depending not only on which information is available, but also on the conclusions drawn from it and the tonnage level.

The quantitative Weight of Evidence approach weighs each available alternative in vitro test or tool for its ability to predict the outcome of the specific golden standard assay (e.g. gene mutations in bacteria).

[Carcinogenicity](#) studies have a qualitative categorical aspect comparable to mutagenicity studies, i.e. they should answer the question whether or not a substance is to be considered carcinogenic, as well as a quantitative continuous aspect comparable to repeated dose studies, i.e. in case the substance is carcinogenic, how potent it is. Studies that are adequate to answer the classification question may not be adequate to answer the potency question, while the reverse is well the case. Since the aim of the ITS for carcinogenicity is to establish whether the available information is sufficient to satisfy the REACH data requirements, the Weight of Evidence approach is limited to the continuous aspect, as the categorical aspect will be implicitly included.

## ITS Bioconcentration Factor

REACH identifies high bioaccumulation potential from the chemicals' bioconcentration factors (BCF) > 2000 (log BCF > 3.3, B chemicals) or > 5000 (log BCF > 3.7, vB chemicals).

The ITS workflow is based on the REACH annexes VII-X, the ECHA guidance on information requirements and chemical safety assessment Chapter R.7c: endpoint specific guidance and Chapter R.11: PBT assessment. All necessary data requirements for the different regulatory purposes C&L, chemical safety assessment (CSA) and assessment of persistence, bioaccumulation and toxicity (PBT) have been taken into account. The suggested scheme explores the REACH regulatory requirements in order to identify test priorities and use of different methodologies such as QSARs, chemical categories, read-across, in vitro and in vivo testing methods for bioconcentration assessment.

Cut-off criteria for substance-specific waiving of experimental BCF studies have been included. A large dataset of experimental BCF was used to test the rules of the waiving scheme: about 60% of the nB compounds were correctly identified as nB without false negatives. The same dataset was also used to verify the ability of the QSAR models to predict BCF. The present tools support to reduce testing for bioaccumulation by ~50% as compared to conventional assessment schemes.

## ITS Aquatic Toxicity

Aquatic toxicity refers to the intrinsic property of a substance to be detrimental to a water organism after short-term and / or long-term exposure. Aquatic risk assessment deals with three major compartments: pelagic, sediment and sewage toxicity. Aquatic pelagic toxicity refers to freshwater and marine organisms living in the water column. In general, it is assumed that aquatic toxicity is mainly related to the



waterborne exposure of a substance and expressed as external concentration of that substance in test water.

The ITS workflow is limited to pelagic toxicity and it is based on the REACH annexes VII-X and the applicable ECHA guidance documents. All necessary data requirements for the different regulatory purposes (C&L, CSA and PBT assessment) have been taken into account. The ITS scheme identifies test priorities and explores the use of QSARs, read-across, in vitro and in vivo testing methods to assess aquatic toxicity.

Screening criteria for the time of degradation and an evaluation of the mode of action of the substances have been included. Tools to obtain information for aquatic toxicity required for the registration of a substance – mainly freely available software or tools developed within OSIRIS – have been added in the ITS scheme.

## Weight of Evidence Approach

The frameworks developed integrate heterogeneous information gathered by several methods, including QSARs, TTC, read-across, in vitro and in vivo tests. These methods are affected by different sources of uncertainty which have to be identified, managed and reduced in subsequent testing cycles by using decision theory tools.

The quantification of uncertainties involves the consideration of probabilities. Bayesian statistics allow the weighting of prior information (including expert information) and information from testing. Moreover, the successive updating of the prediction probability is possible, if new, additional information is introduced. Thus, in a Weight of Evidence (WoE), the result of sequentially adding existing information or generating new information will show whether the confidence in the conclusion has increased. The order in which information from different sources is combined does not influence the calculated posterior probability.

In order to determine how much a single piece of information should contribute to the overall conclusion on the toxicological properties of a substance, the reliability and relevance of that information need to be assessed. This includes a judgement on the reliability and relevance of the individual data and the (scientific) validity of the methods used to generate these data. The different types of endpoints – categorical and continuous – require a different WoE approach. An overall weight factor represents the probability that the collected information will lead to a correct conclusion with respect to the goal it was collected for: classification in case of the categorical endpoints, a reliable potency estimate for the continuous endpoints.

As some of the weight factors have a statistical basis, and others depend to a large extent on expert judgement, a decision framework is needed in which both types of data can be combined. For human toxicological endpoints, dealt within this project, a Bayesian framework was chosen, while for environmental endpoints the Dempster-Shafer theory was preferred.

The Dempster-Shafer theory of evidence is a technique for decision-making under uncertainty which considers sets of hypotheses and assigns probabilities to them. It incorporates complex, even conflicting, information into a mathematical framework. Bayesian analysis is a special case within the Dempster-Shafer theory.

## The OSIRIS Webtool

The methods and ITS developed within OSIRIS for the different human health and environmental endpoints are implemented in the web-based OSIRIS Tool, which will be made freely available for end-users at the end of the project.

The functionalities of the OSIRIS Webtool include:

- Substance entry
- Data entry, with access to integrated databases
- Assessment of information according to endpoints and REACH requirements
- Expert judgement entry
- Decision theory approaches.

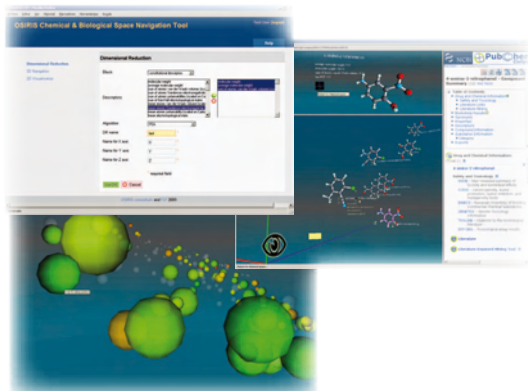
Two uncertainty reasoning schemes are implemented in the ITS: Bayesian Networks and Dempster-Shafer theory of evidence.

The Webtool also includes interfaces to locally installed QSAR software for generating in silico predictions including information about respective applicability domains (e.g. from ChemProp, TIMES etc).

As a result the OSIRIS Webtool indicates what tests (if any) should be performed in order to satisfy REACH data requirements. Data used and decisions taken are documented.



## Chemical Space Navigation Tool



The development of an automated system to implement ITS requires the analysis of a huge amount of chemical data with the purpose of establishing cause and effect relationships or to infer unknown properties based on molecular similarity principles.

A Chemical Space Navigation Tool has been integrated in the OSIRIS Webtool as a visual aid for pre-screening of substances. It is designed to facilitate the exploration of the relationships between chemical structure, physico-chemical properties and environmental endpoints.

### More information is available in the following related OSIRIS publications:

Aldenberg T, Jaworska JS 2010. Multiple test in silico Weight-of-Evidence for toxicological endpoints. In: Cronin MTD, Madden JC (eds): *In Silico Toxicology: Principles and Applications*, Royal Society of Chemistry, Cambridge, UK, pp. 558-583

Jaworska J, Gabbert S, Aldenberg T 2010. Towards optimization of chemical testing under REACH: A Bayesian network approach to Integrated Testing Strategies. *Regul. Toxicol. Pharmacol.* 57 (2-3): 157-167

Fernández A, Rallo R, Giralt F 2009. Uncertainty reduction in environmental data with conflicting information. *Environ. Sci. Technol.* 43 (13): 5001-5006

Vermeire T, van de Bovenkamp M, Bruinen de Bruin Y, Delmaar C, van Engelen J, Escher S, Marquart H, Meijster T 2010. Exposure Based Waiving under REACH. *Reg. Toxicol. Pharmacol.* 58 (3): 408-420

Vonk JA, Benigni R, Hewitt M, Nendza M, Segner H, van de Meent D, Cronin MTD 2009. The use of mechanisms and modes of toxic action in integrated testing strategies: the report and recommendations of a workshop held as part of the European Union OSIRIS Integrated Project. *ATLA* 37: 557-571

Nendza M, Müller M 2010. Screening for low aquatic bioaccumulation (1): Lipinski's Rule of 5' and molecular size. *SAR and QSAR in Environmental Research* 21 (5&6): 495-512

Gabbert S, Weikard H-P 2010. A theory of chemicals testing and regulation. *Natural Resources Forum* 34 (2): 155-164

For a complete OSIRIS publication list see [www.osiris-reach.eu](http://www.osiris-reach.eu) > Publications.

## OSIRIS Methods and Models

OSIRIS developments include:

### Models for toxicity prediction:

- Experimental and computational determination of toxicity-related electrophilicity of chemicals (chemoassays, bioassays, quantum chemistry)
- Prediction of physico-chemical properties and toxicity from structure (ChemProp OSIRIS edition)
- Category formation and read-across approaches
- Prediction of internal exposure
- Prediction of aquatic hazard distribution (NOEC95)

### Models for environmental exposure assessment:

- Bioaccumulation of polar and non-polar compounds
- Fate of neutral and ionisable chemicals
- Fate of parent chemicals and their transformation products
- QSAR predictions of fate-related properties
- Probabilistic exposure assessment

### Screening methods:

- Cut-off criteria for substance-specific waiving of BCF studies
- Screening method for persistent transformation products
- PBT (persistent, bioaccumulative, toxic) index model
- CMR (carcinogenic, mutagenic, reprotoxic) screening tool

### Waiving opportunities:

- Exposure-based waiving (environment, workers, consumers)
- TTC approach for inhalation and dermal exposure
- TTC values for drinking water

### Data collation:

- OSIRIS-wide database system, implemented in ChemProp
- E-SovTox: online database on Russian (eco)toxicity data
- Toxicogenomics data

### Data and model analysis:

- Data quality assessment strategies
- Determination of the applicability domain

### Optimisation possibilities of in vitro tests:

- Passive dosing to control exposure concentrations

### Optimisation possibilities of in vivo tests:

- Guidance on the optimisation of in vivo tests
- Acute-chronic ratios to prioritise chemicals for chronic aquatic toxicity testing
- Data quality-related optimisation of testing protocols, in vitro and in silico approaches for ecotoxicity testing

### Decision models:

- Cost-effectiveness analysis model
- Value-of-Information (VOI) model for sequential testing

### ITS acceptance:

- Survey on ITS implementation and acceptance

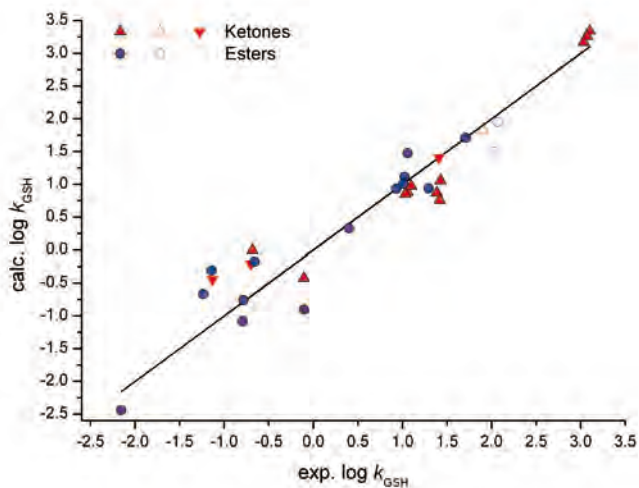
## Chemoassay to quantify electrophilic reactivity (Partner UFZ)

A new approach was established to quantify the electrophilic reactivity of compounds as trigger of their reactive toxicity. To this end, a kinetic chemoassay employing glutathione (GSH) as soft model nucleophile was developed, and applied to 46  $\alpha,\beta$ -unsaturated carbonyls (15 ketones, 16 esters, and 15 aldehydes). The goal was to introduce a reactivity parameter that can be used both for directly quantifying the reactive component of the toxicity of respective electrophiles, and for building a reactivity scale as basis for developing computational chemistry models of electrophilic reactivity.

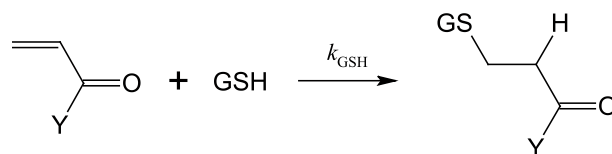
As compared to a previously introduced static variant, the kinetic GSH assay is significantly more sensitive at both the low and high end of reactivity, and also better addresses confounding factors, e.g. GSH loss due to oxidation.

For the actual dataset of 46  $\alpha,\beta$ -unsaturated carbonyls, multiple regressions employing both electrophilic reactivity in terms of the logarithmic 2<sup>nd</sup> order rate constant,  $\log k_{\text{GSH}}$ , and compounds' lipophilicity given by calculated octanol/water partition coefficients ( $\log K_{\text{ow}}$ ) yield good correlations with the toxicity towards *Tetrahymena pyriformis* (48-h 50% growth inhibition) for subsets of ketones ( $r^2 = 0.96$ ), esters ( $r^2 = 0.91$ ), and aldehydes ( $r^2 = 0.96$ ). Moreover, for the first time it has been shown that within the Michael acceptor domain systematic differences in chemical structure between ketones, esters, and aldehydes translate into different contributions of reactivity and lipophilicity to compounds' aquatic toxicity.

For future work these data will form a basis for developing computational chemistry tools to predict local molecular electrophilicity from molecular structure.



$\log k_{\text{GSH}}$  of 31  $\alpha,\beta$ -unsaturated carbonyl compounds calculated using  $\omega_{\text{O},\text{BC}}^q$  versus the experimental values. Circular symbols represent esters, triangular symbols ketones, with cyclic compounds indicated through downward triangles. Filled symbols refer to Michael systems with a C=C double bond, open symbols to C=C-C=O systems. The solid line indicates identity.



1,4-Michael addition of GSH to an  $\alpha,\beta$ -unsaturated ketone (Y = alkyl), ester (Y = OR), or aldehyde (Y = H).

Böhme A, Thaens D, Paschke A, Schüürmann G 2009. Kinetic glutathione chemoassay to quantify thiol reactivity of organic electrophiles – Application to  $\alpha,\beta$ -unsaturated ketones, acrylates, and propiolates. *Chem. Res. Toxicol.* 22(4): 742-750

Böhme A, Thaens D, Schramm F, Paschke A, Schüürmann G 2010. Thiol reactivity and its impact on the ciliate toxicity of  $\alpha,\beta$ -unsaturated aldehydes, ketones, and esters. *Chem. Res. Toxicol.* 23(12): 1905-1912

## Prediction of the toxicity-relevant reactivity by local electrophilicity (Partner UFZ)

Electrophilic substances are able to form covalent bonds to nucleophilic reaction sites in proteins and DNA. This results in reactive toxicity and associated diseases such as dermal or respiratory sensitisation and mutagenicity. For this reason, the prediction of the reactive behaviour of electrophilic compounds as potential reactive toxicants is of great importance for the risk assessment of chemicals, e.g. in the context of the EU regulation for industrial chemicals REACH. In silico approaches as alternative methods for hazard and risk assessment could facilitate the evaluation of chemical substances.

In view of a quantitative prediction of electrophilicity based on the molecular structure, two new local electrophilicity parameters  $\omega_{r,s}^q$  and  $\omega_{r,s}^E$  were derived, using site-specific quantum chemical parameters for the quantification of the energy change associated with the gain or loss of electronic charge, since these reactions are driven by the electron transfer from the nucleophile to the electrophile.

Both local electrophilicity parameters showed superior behaviour within two test cases compared to previous approaches such as Parr's global electrophilicity  $\omega$  or its local variant  $\omega_r^+$  using condensed-to-atom Fukui functions.

In the first test case both parameters were used to predict experimental reaction rate constants of a set of 31  $\alpha,\beta$ -unsaturated carbonyl compounds towards the model nucleophile glutathione, an antioxidant protecting cells from reactive electrophilic species. As glutathione is highly available within the cytoplasm, it is supposed to react with most electrophiles in the first place, thereby protecting proteins and DNA up to the point at which its concentration is critically decreased. We were able to reproduce the logarithmic experimental reaction rate constants of the test set consisting of 15 ketones and 16 esters via multilinear least squares regression yielding  $r^2$  (squared correlation coefficient) values up to 0.95.

The second data set demonstrates the suitability of the new



reactivity parameters to model Mayr's electrophilicity parameter  $E$  for 20 benzhydrylium cations by yielding  $r^2$  values up to 0.99. The parameter  $E$  forms the basis of an experimentally derived electrophilicity scale and is used in combination with two nucleophile dependent parameters to compute reaction rate constants for electrophile–nucleophile reactions. The experimental determination of these three parameters is difficult, hence they are available only for the minority of toxicologically relevant substances. In this context, the prediction of the parameter  $E$  is a promising first step towards the extension of Mayr's electrophilicity scale without further need of experimental work.

Both results indicate the suitability of the two new local electrophilicity parameters to screen organic compounds *in silico* for their electrophilic reactivity in general, and for their potential to exert reactive toxicity in particular.

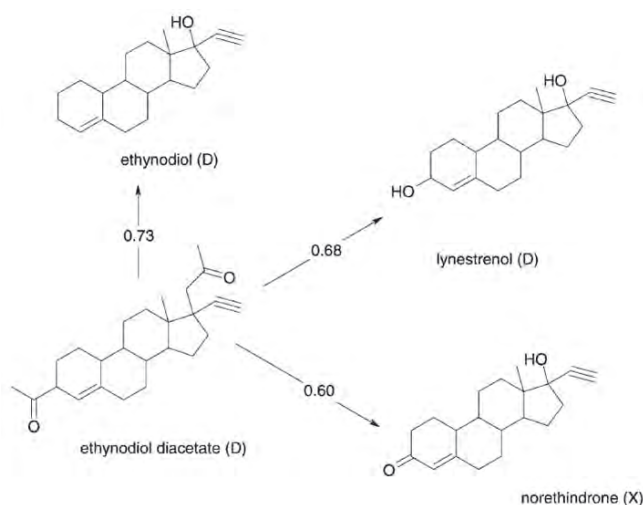
Wondrousch D, Böhme A, Thaens D, Ost N, Schüürmann G 2010. Local electrophilicity predicts toxicity-relevant reactivity of Michael acceptors. *J. Phys. Chem. Lett.* 1: 1605–1610

## Formation of categories for read-across of endpoints relating to reproductive toxicity (Partner LJMU)

The use of category formation and read-across to predict endpoints relevant to reproductive toxicity – one of the major endpoints for REACH – has been illustrated. Category formation is one of the key *in silico* methods being developed in the OSIRIS project. This technique is one of the most applicable methods to make predictions of complex human health endpoints. Once a robust category has been formed i.e. a grouping of chemicals that are related on some rational basis, toxicological data can be found, interpolation or read-across can be performed to make predictions.

The OSIRIS project is developing many approaches to form categories to predict toxicity. Amongst these, those based on modes and mechanisms of toxic action are preferred. However, these approaches are applicable only if a mode or mechanism is known. For complex endpoints, such as reproductive toxicity, information on modes and mechanisms is often lacking. Therefore, a novel approach applied in the OSIRIS project has been to use 2D measures of molecular similarity to form categories.

The freely available Toxmatch software, developed by the European Commission's Joint Research Centre (JRC – an OSIRIS partner), Ispra, Italy was applied to form categories of molecules. To illustrate its use, a data set of teratogenicity values for chemicals with many different (and mostly unknown) mechanisms was investigated. Categories of structurally similar chemicals were created which allowed for read-across to make predictions for compounds excluded from the training set i.e. a true estimation of predictivity. It was concluded that 2D



similarity methods offer a useful method for building chemical categories for teratogenicity in which a priori mechanistic knowledge is limited. The study has provided a valuable insight into strategies to create “similarity-based” categories and use them to make read-across predictions of toxicity for complex human health endpoints.

A simple example of such a category (the numbers are the level of similarity on a scale of 0 (completely dissimilar) to 1 (identical)) is shown in the figure. The consensus from the read-across within this category is the correct prediction of the query chemical (ethynodiol diacetate) to be teratogenic.

Enoch SJ, Cronin MTD, Madden JC, Hewitt M 2009. Formation of structural categories to allow for read-across for teratogenicity. *QSAR and Combinatorial Science* 28: 696-708

## Model development for internal exposure (Partners CYPOTEX, TNO)

Prediction of human internal exposure to chemicals arising from external exposure is performed by means of a physiologically based pharmacokinetic (PBPK) model. For this purpose, models including mathematical descriptions of the physiological processes in the body are developed for the simulation of absorption, distribution, metabolism and elimination (ADME) of compounds in both rats and humans.

The models are developed to predict internal exposure after absorption via oral, dermal or inhalative exposure to REACH compounds, or following intravenous injection, and are designed to predict the internal exposure in rats and for humans. The similarity of the underlying models for rat and human enables the predictions for rat to be used as a surrogate for predictions for human exposure – necessary because of the difficulty of obtaining good quality datasets for human exposure for REACH-relevant compounds.

In general, the compound-specific information for these

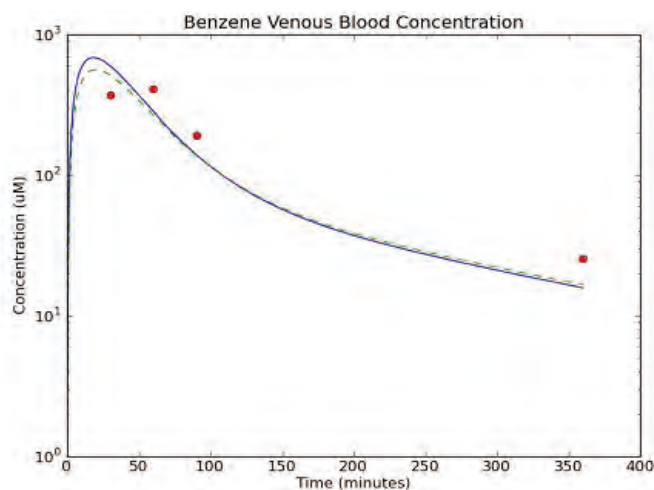
chemicals is limited as compared to the pharmaceuticals compounds for which originally physiologically based models are designed. Therefore, a generic kinetic model (i.e. a model that can be applied to a wide range of compounds, rather than one, or a few related compounds) is designed and where needed compound-specific information is either measured in vitro or predicted using QSARs. A third option is to use default values for certain parameters for which no measured or predicted values are available.

A preliminary validation of the PBPK models developed has been carried out. Predictions have been performed for seven compounds, introduced via one or more routes (oral, pulmonary, dermal and/or intravenous injection) in human or rats, and compared to existing in vivo data. The preliminary investigation of the utility of generic PBPK models for the prediction of toxicokinetics (TK) of REACH-relevant compounds has illustrated that reasonable predictions are achievable.

For the purposes of OSIRIS, it is planned that TK prediction will be incorporated into exposure-based waiving for the Integrated Testing Strategies developed for human health endpoints. The key TK parameters of amount absorbed (for oral, pulmonary and dermal exposure routes) and half-life will be predicted. Consequently, further validation of the models' utility for incorporation in ITS will focus on the reliability of predicting (1) amount absorbed and (2) half-life.

Particular attention will be paid to the chemical domain within which the predictions are expected to be valid. For half-life, this requires particular attention to be paid to determining whether or not all means of elimination have been identified. It also requires that chemistry-specific tissue binding over and above that which can be predicted from generic partitioning can be accounted for within the ITS.

Metcalfe PD, Thomas S 2010. Challenges in the prediction and modelling of oral absorption and bioavailability. *Curr. Opin Drug Disc. Devel.* 13: 104-110



Predicted and observed blood concentrations for oral administration of benzene to adult male Wistar rats. Red circles: measured in vivo concentrations; blue solid line: predicted concentration in the bulk venous plasma; green dashed line: predicted concentration at the sample site.

## Recommendations for the use of toxicological and mechanism of action information to support informed hazard assessment (Partners RIVM, LJMU, ISS, AL, UB)

A full report has been published in ATLA as part of a special edition on in silico techniques edited by Mark Cronin, Liverpool John Moores University on an influential OSIRIS Workshop that has made recommendations for the use of toxicological mode and mechanism of action information to support informed hazard assessment:

Vonk JA, Benigni R, Hewitt M, Nendza M, Segner H, van de Meent D, Cronin MTD 2009. The use of mechanisms and modes of toxic action in integrated testing strategies: the report and recommendations of a workshop held as part of the European Union OSIRIS Integrated Project. *Alternatives to Laboratory Animals* 37: 557–571.

The Workshop was held in Liverpool, England on 30 October 2008, being attended by over 35 delegates including experts from OSIRIS and key invited external leaders in the field. The aim of the Workshop was to build upon recent progress in this area from the US EPA, the International Programme for Chemical Safety (IPCS) and other groups.

The delegates first set about defining the terms mode and mechanism of action. A clear and obvious problem here is that researchers from different backgrounds (e.g. human health vs environmental) attached different meanings to these terms. It was agreed that loose definitions are required; it is the context of using this information that is more important. Delegates then split into three groups to discuss the role of modes and mechanisms of action for environmental toxicity, local human effects and chronic human toxicity.

The findings of the workshop clearly shape the state of the art into a usable form. There was overwhelming agreement that if information on modes and mechanisms of action can be captured, then this information can be used to group chemicals into pragmatic categories. For instance, for environmental toxicity, the role of mechanistically based computational models for acute toxicity is well established – a key factor is whether a compound can be classified as being narcotic. This goes beyond acute toxicity, as the workshop concluded that acute-chronic ratios could be better applied within a mechanistic framework, i.e. the extrapolation is more reliable for narcotics. There are a number of methods of assigning mechanisms of action (e.g. the Verhaar rules) which are being developed and implemented in the OSIRIS project.

For human health effects, the Workshop focussed on skin sensitisation (a local effect) and carcinogenicity. There is extensive mechanistic understanding of both endpoints.



For the important REACH endpoint of skin sensitisation, the immunological pathway leading to a response has been well established. The focus of modes and mechanisms of action is to capture knowledge on electrophilic chemistry which is the initiating event. This can be used as a direct predictor of toxicity or a method to group chemicals together. For carcinogenicity, consideration was given to genotoxic and non-genotoxic mechanisms. Genotoxic mechanisms can be captured with structural fragments, such information is already implemented in OSIRIS. Non-genotoxic mechanisms are more complex to understand and are an area of further development. One way in which OSIRIS is supporting all these activities is in the development of high quality databases for these endpoints.

The Workshop was able to form a consensus on all aspects. This is summarised in the report which describes the key knowledge on how to group chemicals on a mechanistic basis. This includes the state of the art of the techniques to achieve this. Many of these are implemented in, and will guide, the OSIRIS Integrated Testing Strategies and will make progress in the reduction of the use of animals for toxicological assessment.

### ChemProp, an in silico tool for prediction of chemicals' properties and toxicity (Partner UFZ)

Developed by OSIRIS partner UFZ, the software system ChemProp predicts compound properties from chemical structures by means of qualitative/quantitative structure-activity relationships (QSARs) and contains databases with compound properties.

The database module supports structure searching in external SQL resources (typically Excel files) and in WWW resources (via eMolecules). Substructure searching facilities are implemented for internal and external resources, together with a graphical substructure query editor. The database currently contains ca. 15,000 entries including conformers and specific tautomers, covering more than 10,000 different chemicals. OSIRIS datasets

of (eco-)toxicological test results are accessible as external databases via ChemProp.

ChemProp includes QSAR methods for physico-chemical, ecotoxicological and toxicological endpoints. The OSIRIS edition of the ChemProp software is currently offering ca. 80 models for predicting about 40 different properties regarding partitioning, degradation, environmental fate, ecotoxicology and toxicology. Read-across models based on atom-centred fragments (ACF) and characterisation of the applicability domain with particular respect to the chemical domain by means of ACF are included.

Compounds can be imported via SMILES, existing files, e.g. in .SDF or .XML format, a graphical editor or by searching the ChemProp databases. The results of the detailed compound profiling are automatically summarised in a report for later documentation.

### Bioaccumulation model for polar and non-polar compounds (Partner SU)

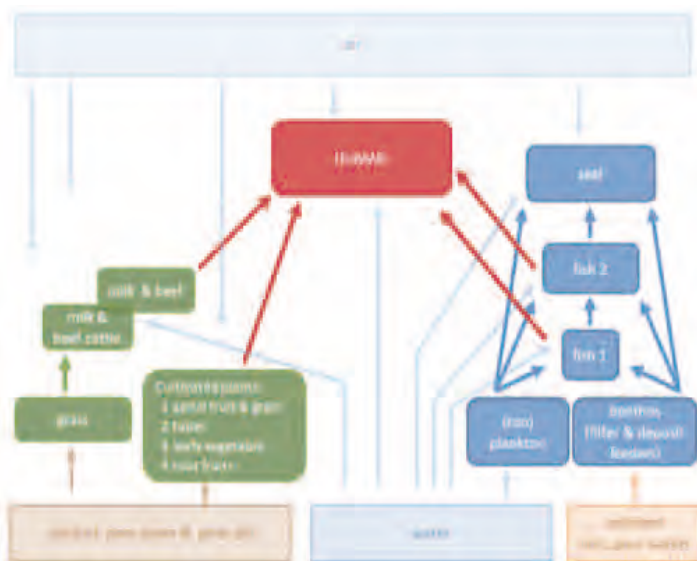
A bioaccumulation model for polar and non-polar compounds was developed assembling state-of-the art approaches in exposure modelling including mechanistic process descriptions and quantification of phase partitioning with polyparameter linear free energy relationships (pp-LFER). The latter enables an extension of the range of application of the model from neutral non-polar chemicals (the domain of existing bioaccumulation models) to neutral polar compounds. The model includes modules of uptake into plant developed by the OSIRIS partner DTU.

The model is designed as a hybrid of a steady state and a non-steady state model. It is subdivided into an aquatic food web, an agricultural food chain, four different types of crops/cultivated plants, and the human as the top consumer and model endpoint. Bioaccumulation in the long-living carnivorous top predators seal and human is described with a non-steady state approach, whereas the lower trophic levels are assumed to be in a steady-state.

In addition to dietary uptake, the model considers direct exposure to contaminants present in the physical environment via respiration, drinking and ingestion of soil or sediment particles. Elimination pathways considered for each organism are excretion via feces, respiration, metabolism, growth, and for mammals additionally urination, percutaneous excretion, and in the case of females, loss via birth and lactation. For the vegetation, contaminant uptake from both soil and the atmosphere is considered, as well as contaminant loss due to biotransformation, due to growth, and loss to the atmosphere.

The model can be easily linked to multimedia fate and transport models, allowing a sophisticated estimation of the contaminants distribution in the environment and the exposure of wildlife and humans as the result of chemical emissions. Alternatively, the user can directly define environmental concentrations or fugacities which are used as input for the





Overview scheme of the bioaccumulation model (Gertje Czub)

bioaccumulation model. For this purpose, a tool is provided to calculate environmental fugacities from given concentrations or, alternatively, from a level I unit world, consisting of an air compartment including aerosols, a water compartment including suspended matter, a sediment compartment, and a soil compartment. The default parameterisation is set in accordance with the regional default scenario of EUSES.

Undeman E, Czub G, McLachlan M 2009. Addressing temporal variability when modeling bioaccumulation in plants. *Environ. Sci. Technol.* 43 (10): 3751-3756

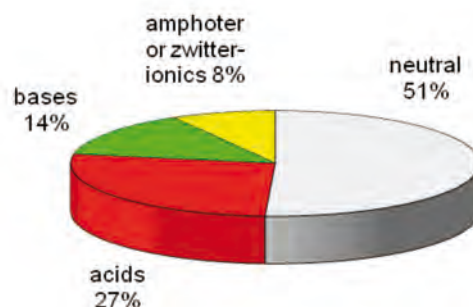
## Regional dynamic model for ionisable compounds (electrolytes) (Partner DTU)

The diversity of compound classes within the REACH chemical space represents a major challenge for risk assessors. Ionisable organic groups such as carboxylic acids, phenols, amines and anilines, are frequent in many organic industrial chemicals. A particular class of ionisable organics are ionic surfactants, very frequent in detergents, dyes, pigments, adhesives and other products. To better characterise the poorly known occurrence of ionisable organics among industrial chemicals, a screening study was performed on a representative sample of substances pre-registered to REACH. A representative random sample (1.5% out of the approximately 117 000 substances due to registration in 2010 and 2013) was selected and processed using the software ACD/Labs® to calculate the dissociation constant(s) ( $pK_a$ ), the octanol-water partition coefficient of the neutral molecule ( $\log K_{ow}$ ) and the vapour pressure of the neutral molecule ( $p_v$ ).

Almost one half of the screened compounds are at least partially ionised under environmentally relevant conditions (pH 4 to 10) (see figure). Among these, most are acids (27%), but also bases

(14%), amphoters and zwitterionics (8%, molecules including both acidic and basic groups) are common. Most substances have  $\log K_{ow}$  ranging between 0 and 4. Hydrophilic chemicals are most frequent (30% with  $\log K_{ow} < 1$ ) but super-hydrophobic chemicals are present as well (10% with  $\log K_{ow} > 6$ ). About 28% of these very super-hydrophobic, i.e. 3% of the total sample analysed, are mostly ionised at pH 7. Long lipophilic structures with an ionisable head (e.g. surfactants) fall into this category. REACH chemicals generally exert low vapour pressure:  $p_s < 1$  Pa for 65% and  $> 100$  Pa for only 13%. The apparent volatility may be even lower due to ionisation, because the vapour pressure of ionic species is negligible.

The results of this screening study highlight the need to extend the applicability domain of existing models and refine model predictions, taking into account the effect of pH.



Percentage of ionics from 1510 pre-registered REACH chemicals. Only acid  $pK_a < 12$  and basic  $pK_b > 2$  are considered.

The environmental exposure of organisms and humans to chemicals via air, water, soil and food is implemented in the EU tool for the chemical safety assessment (EUSES). The fate of a chemical released to the environment is predicted via a regional level III multimedia model (SimpleBox), local models (for water, air and soil systems) and food chain models. These models are based on algorithms and exposure pathways that were developed for neutral and lipophilic chemicals. In many cases they are not valid for polar and ionisable chemicals.

Therefore a multimedia environmental exposure-model applicable for both neutral and ionisable chemicals has been developed from the regional unit environment implemented in SimpleBox: the level III-IV Multimedia Activity Model for ionisable chemicals (MAMI III-IV).

MAMI is based on the chemical activity replacing the traditional approach based on fugacity or concentration. The chemical activity, or apparent concentration in water, is a convenient model variable as it can be readily defined for neutral and ionic species. The activity approach introduces a new quantity named "activity capacity" to link activities to concentrations.

Chemical equilibrium is defined by equal activities. Diffusion is driven by activity gradients while intermedia advective transport and transformation processes are described by the total concentration. The distribution between neutral and ionic species is described by the Henderson-Hasselbalch equation. Equilibrium between phases is described by species-specific partition coefficients.

The distribution between compartments depends largely on the dissociation status (and therefore the pH) of ionisable compounds, and on the transfer rates of the various species (neutral, ions).

Franco A, Ferranti A, Davidsen C, Trapp S 2010. An unexpected challenge: ionizable compounds in the REACH chemical space. *Int. J. LCA* 15: 321–332

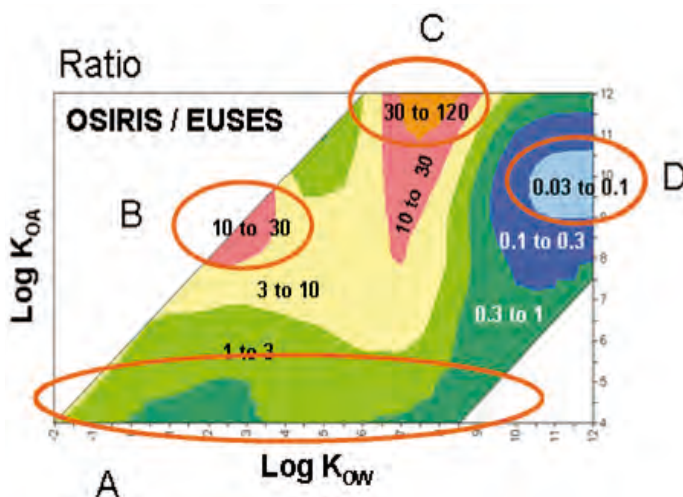
Franco A, Trapp S 2010. A multimedia activity model for ionizable chemicals: validation study with 2,4-dichlorophenoxyacetic acid, aniline and trimethoprim. *Environ. Toxicol. Chem.* 29(4): 789–799

Trapp S, Franco A, Mackay D 2010. Activity-based concept for transport and partitioning of ionizing organics. *Environ. Sci. Technol.* 44(16): 6123–6129

## Regional model for parent compounds and transformation products (Partner ETHZ)

Degradation products can make a significant contribution to the overall persistence and longrange transport potential of certain chemicals. Therefore, the inclusion of transformation products within the exposure assessment framework should reduce the likelihood of false negatives, i.e. chemicals designated to pose “low risk of exposure” but whose transformation products persist in the environment, thereby contributing to the overall exposure level associated with the release of the parent chemical.

ETHZ developed a level III regional exposure model that simultaneously calculates environmental concentrations of parent compounds and of their degradation products. This prototype model includes a regional box model based on



Ratio between the daily dose (kg chemical kg<sup>-1</sup> body weight) estimated by OSIRIS and EUSES (DoseOSIRIS/DoseEUSES), plotted as a function of the log octanol-air ( $K_{OA}$ ) and log octanol-water ( $K_{OW}$ ) partition coefficients, assuming emissions to air only and no biotransformation.

SimpleBox 2.0 and a municipal sewage treatment plant model based on SimpleTreat 3.0. The model includes the sewage treatment plant as an important site of biodegradation, and therefore as an important generation site for degradation products. The model is programmed in Matlab and in Python.

The key purposes of this prototype model are:

- proof of concept for simultaneously modelling parent compounds and their transformation products in a regional environmental exposure model and
- means of identifying data needs and current data gaps for successfully implementing such a model.

## Comparison of OSIRIS models to the EU regional model for environmental exposure (EUSES) (Partners SU, DTU, ETHZ)

### Comparison of models for bioaccumulation

An spLFER (single parameter linear free energy relationship) version of the OSIRIS model for bioaccumulation (SU) was compared to the EUSES model (also based on spLFERs). The models predict the total daily dose of a chemical to a human based on the chemical's concentrations in air, water, soil, and sediment. Total daily doses were estimated for hypothetical persistent chemicals (i.e. no biotransformation) assuming equilibrium partitioning between air, water, soil and sediment.

The model comparison identified regions of the chemical partitioning space where i) the two models generated similar results (A) ii) the OSIRIS model generated substantially higher daily doses (B, C) and iii) the OSIRIS model generated substantially lower daily doses (D) (see figure). The comparison shows that the OSIRIS model in general predicts significantly higher human daily doses for all but the super hydrophobic chemicals. The difference between the models was particularly striking for high  $K_{OW}$  and high  $K_{OA}$  compounds due to significantly higher concentrations in fish predicted by OSIRIS, and the very high concentrations in root crops predicted by EUSES. The regression employed in EUSES to calculate uptake of chemicals from soil yielded a comparatively low uptake of hydrophilic compounds in vegetation, which in turn resulted in lower intake via this exposure vector than in the OSIRIS model.

### Comparison of new multispecies models to the single-species EUSES model

The Multimedia Activity Model for Ionisable chemicals (MAMI, DTU) is designed to predict the environmental fate of neutral and ionisable chemicals, the Multi-Species Multi-Media model (MS-MM, ETHZ) includes the fate of degradation products that result from the breakdown of an emitted chemical in the



environment. A single-species model identical to the regional scale of SimpleBox (the exposure model implemented in EUSES) was developed as reference.

The comparison of MAMI with EUSES highlighted the impact of dissociation and pH on the partitioning constants of ionisable substances. The species specific estimations for the  $K_{oc}$  in MAMI differ remarkably from the regressions implemented in the EUSES regional model. The latter neglect the impact of pH and electrical interactions on the sorption to solids and likely underestimate PECs of organic bases in soil and sediments.

For the MS-MM model, the most important impacts were on determination of chemical persistence – including degradation products would increase the number of substances classified as persistent and very persistent under REACH –, the choice of whether testing should consider single-chemical or multi-component (mixture) toxicity, and whether degradation products could trigger tests, such as sediment or soil toxicity tests that would not be triggered by the parent compound alone.

The degradation pathway of organic chemicals often includes ionisable metabolites. The combined use of the MS-MM model for degradation products and the activity model for neutral and ionisable chemicals MAMI provides a robust model framework for high tier exposure assessment of multispecies chemicals.

Such a combined use may indeed be necessary given that the fate of ionisable metabolites that are persistent and accumulate in soil and sediment compartments may dominate aspects of chemical classification (based, e.g., on persistence) and on the waiving or triggering of specific tests (e.g. whether a sediment toxicity test is warranted).

## Refined cut-off criteria for substance-specific waiving of bioassays (Partner AL)

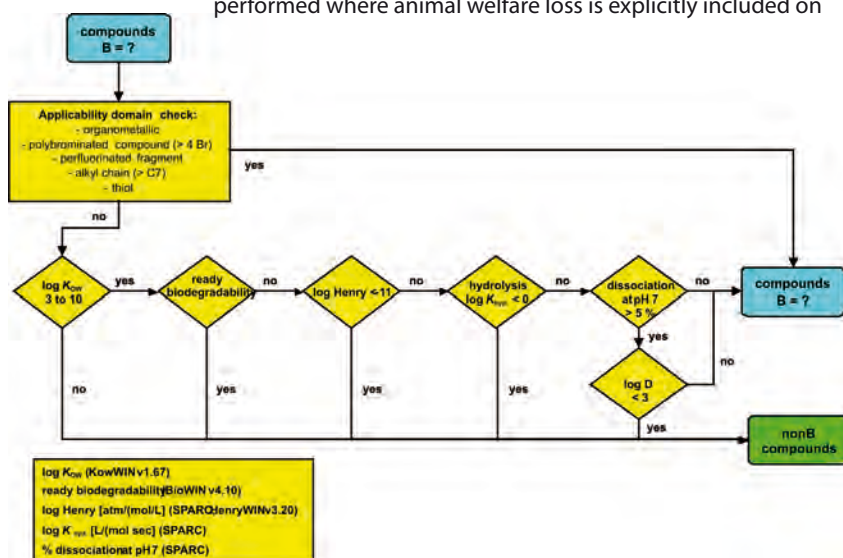
According to REACH, substances of very high concern, such as (very) persistent, (very) bioaccumulative and toxic (PBT, vPvB) chemicals require authorisation, and their use may be restricted. REACH identifies high bioaccumulation potential from the chemicals' bioconcentration factors (BCF) > 2000 (log BCF > 3.3, B chemicals) or > 5000 (log BCF > 3.7, vB chemicals).

The aim of the present work is to protectively de-prioritise nonB compounds with BCF < 2000. Major emphasis is put on 'safe' criteria, excluding false negatives. Eventually, experimental BCF studies for chemicals with bioavailability constraints may be waived because they either provide no risk-relevant information or are unworkable to perform. So far, bioconcentration cut-off criteria have been focussed on molecular size, assuming that membrane permeation of

large molecules is limited. However, no robust evidence was found for cut-offs in bioconcentration related to molecular size. Rather, a modulating effect of molecular size on membrane permeation appears to exist. Moreover, the ensemble of molecular attributes according to Lipinski's 'Rule of 5' was found to be inadequate to identify nonB compounds.<sup>1</sup> Possible reasons are differences in the dominating processes during oral absorption of pharmaceutical drugs (bulk dissolution) and the uptake of waterborne environmental contaminants by aquatic organisms (continuous low-level exposure).

The current BCF waiving scheme is based on physico-chemical properties related to mediaspecific exposures and bioavailability. The primary logic is that only if a compound is present in the water in any form (determinants: water solubility, degradability, vapour pressure), it may be taken up by organisms (determinants: log  $K_{ow}$ ,  $pK_a$ ). Protective screening criteria in lipophilicity, ionisation, Henry's law constant (presumably combining information about water solubility and volatility) and stability in water phases (in terms of hydrolysis and ready biodegradability), see figure) reliably identify ~50% nonB compounds (BCF < 2000). If polybrominated compounds (> 4 Br), organometallics, compounds with perfluorinated fragments, substances with an acyclic alkyl moiety (chain length > C7) and thiols are excluded from the applicability domain, no false negatives have been detected (sensitivity of 100 %).<sup>2</sup>

The ongoing update accommodates notorious problem compounds like pentachlorophenol and triclosan. It further elaborates on the definition of applicability domains: The structural domain definition excludes chemical classes of false negative outliers and applies atom centred fragments (ACF) to characterise substances (in)correctly classified. Physico-chemical property ranges and characteristic combinations of physico-chemical properties discriminate either nonB or B compounds. Bayesian statistics are used to determine the probability that a predicted classification is true, given an imperfect Golden Standard with missing or wrong/highly variable data. To conclude, a cost-effectiveness analysis is being performed where animal welfare loss is explicitly included on



Workflow of the BCF waiving scheme based on physico-chemical properties.

the cost side. This allows for a more balanced evaluation of the BCF waiving scheme compared to standard BCF studies.

## References

- ECHA 2008. Guidance on information requirements and chemical safety assessment, Chapter R.11: PBT assessment
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23: 3-25
- University of Georgia 2002. SPARC on-line calculator. <http://ibmlc2.chem.uga.edu/sparc/>
- US EPA 2009. EPI Suite v4.0. <http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm>

<sup>1</sup> Nendza M, Müller M 2010. Screening for low aquatic bioaccumulation (1): Lipinski's 'Rule of 5' and molecular size. *SAR and QSAR in Environmental Research* 21 (5&6): 495-512

<sup>2</sup> Nendza M, Herbst T 2011. Screening for low aquatic bioaccumulation (2): Physico-chemical constraints. *SAR and QSAR in Environmental Research* 22 (1&2): in press

## A screening method for transformation product persistence (Partner ETHZ)

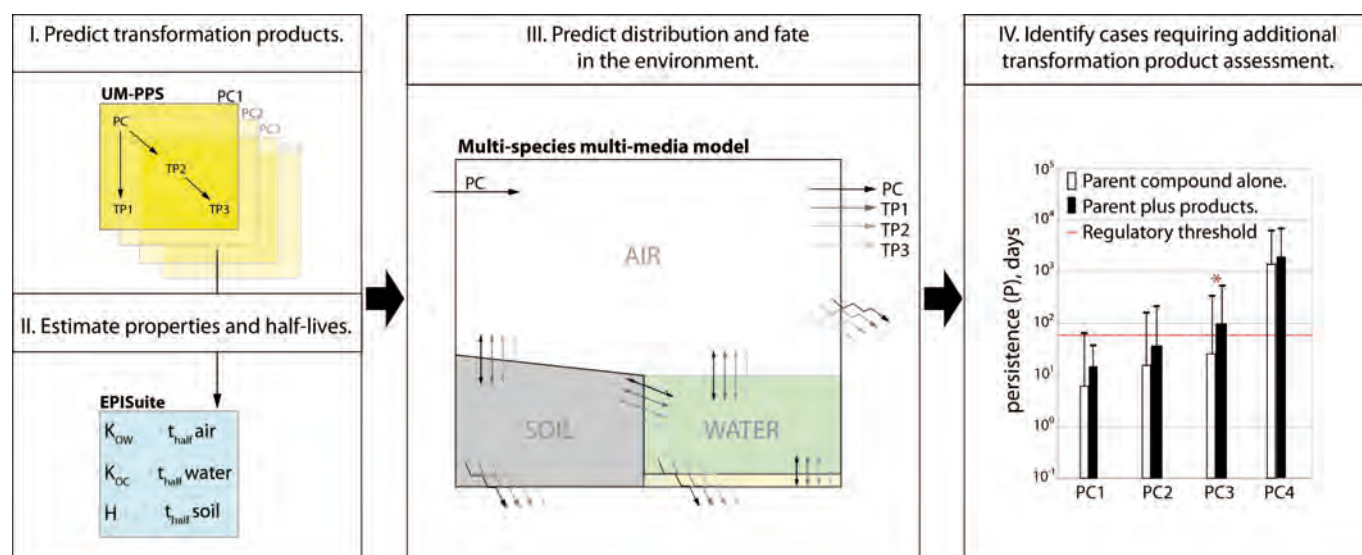
REACH requires that 'significant' transformation products be included in assessments for chemicals produced or imported at more than 100 tonnes per year. The implementation of this requirement will be extremely challenging, due to the lack of available data and reliable quantitative-structure activity relationships (QSARs) for the ill-defined chemical space of degradation products. As part of the OSIRIS project's evaluation of environmental exposure assessment under REACH, ETH Zurich has been working on models that simultaneously treat the fate of parent (emitted) chemicals and their transformation products in the environment. However, application of such models presupposes the availability of property and degradation data for parent chemicals (PCs) and transformation products (TPs). The costs associated

with the assessment of PCs alone (in terms of both money and test organisms) are already high, and the inclusion of an unspecified number of possibly important TPs could increase them exponentially. Thus, a procedure to screen for potentially important TPs prior to testing is highly desirable.

We have constructed a preliminary scheme to assess whether effective transformation product screening can be performed given current data limitations. It consists of four main elements: (i) prediction of TPs for a given PC; (ii) estimation of physico-chemical properties and degradation rates for the PC and its TPs; (iii) prediction of mass distributions in the environment, from which persistence can be calculated; (iv) comparison of predicted persistence, with and without the inclusion of TPs, to relevant thresholds (e.g. 60 day half-life).

The goal of the screening scheme is to identify for which chemicals the inclusion of TPs in persistence estimates would change the classification of the PC from non-persistent to persistent. To evaluate our screening scheme, we chose 22 test cases for which biodegradation pathways are known and compared their classification with and without TPs to persistence classifications based on predicted products with estimated properties and half-lives. We included uncertainties around property and half-life estimates. Our scheme was able to identify the 8 cases out of 22 for which inclusion of TPs in persistence calculations could affect classification relative to a typical threshold half-life of 60 days. However, classification itself would not be possible using this scheme due to very high uncertainty with respect to media-specific half-lives and, to a lesser extent, physico-chemical properties like the Henry's law coefficient. Our scheme provides a starting point for the prioritisation of further experimental work that is sorely needed to expand our knowledge about and confidence in degradation and partitioning properties for industrial chemicals and their transformation products.

Ng CA, Scheringer M, Fenner K, Hungerbühler K 2011. A Framework for evaluating the contribution of transformation products to chemical persistence in the environment. *Env. Sci. Technol.* 45: 111-117



## Exposure based waiving under REACH (Partners RIVM, TNO, FhG)

Within the REACH framework, but also within OECD, there is understanding that for reasons of animal welfare, costs and logistics, it is important to limit the number of tests to be conducted. Exposure based waiving (EBW) is a potentially important element in alternative testing strategies. In a recent publication, the criteria for exposure based waiving as foreseen in the REACH regulation have been described and more detail to the REACH requirements for EBW has been given.

The principle behind any EBW is that there are situations when human or environmental exposures are so low or infrequent that there is a very low probability that the acquisition of additional effect information may lead to an improvement in the ability to manage risk. EBW therefore is risk based and needs thorough knowledge on exposure as well as on effects criteria. Exposure models have been analysed and the uncertainty in their predictions has been discussed as well as no-effect criteria such as the Threshold of Toxicological Concern, termed no-further-action level (NFAL). Examples of EBW have been provided for environmental, consumer and worker exposure.

REACH only allows EBW in a limited number of cases with constraints on tonnage levels, types of tests to be waived and the need for a thorough Exposure Scenario (ES) and exposure assessment throughout the life cycle of a chemical and for all human exposure routes and environmental pathways. EBW will only be considered a real option by industry if a cost-benefit analysis shows an advantage, which may heavily depend on the weighing factor one applies for the non-use of experimental animals.

For many substances, Predicted No Effect Levels (PNECs) and Derived No Effect Levels (DNELs) will not be available as NFALs. For such data-poor substances the concept of Threshold of Toxicological Concern (TTC) has been proposed as a pragmatic approach to establish the exposure level below which no adverse effects on human health or an environment ecosystem are expected to occur. TTCs are derived for structural classes of substances by analysing the distribution of NOELs from in vivo studies. To apply the TTC concept, information about

the chemical structure of the substance, but not toxicological information, is prerequisite.

The possible application of EBW for aquatic toxicity tests has been exemplified on the basis of the substance dibutylphthalate (DBP) using the EXCEL spreadsheet version of EUSES with the add-in Crystal Ball installed. The example concerns the production/formulation of adhesives including DBP with a total estimated tonnage level in the EU produced of 100 kg / annum. Corresponding emission characteristics and other used input distributions, i.e., physical and chemical properties (like water solubility and vapour pressure, etc.) and degradation and transformation rates (like degradation of DBP in surface water, air and sediment, etc.) were used and inserted into EUSES. By running EUSES with 2000 iterations (latin hypercube sampling) a Predicted Environmental Concentration (PEC) distribution of DBP in fresh water was derived as illustrated in the figure.

The figure illustrates the distribution (histogram and best fit overlay) of the simulated PECs in fresh waters. Assuming that the Threshold of No Concern for freshwater systems (ETNCAq) for organic chemicals of 0.1 µg/L is the best estimate representing a NFAL for surface water, it can be seen that the TTC level is exceeded at the 26<sup>th</sup> percentile of the PEC distribution. EBW would not be justifiable. However, if the PNEC of 10 µg/L for DBP from the EC Risk Assessment Report 2003 is used as NFAL, the PEC distribution is below this level and EBW is justifiable. Specific PNEC-values are to be preferred above generic TTCs.

Similar examples were developed for EBW-analyses for consumer and worker exposure.

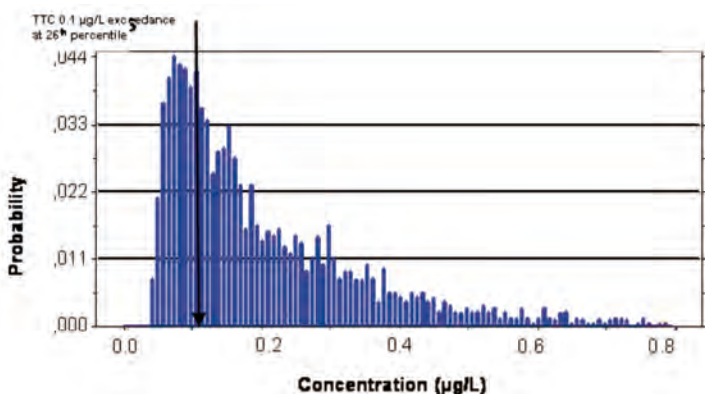
Vermeire T, van de Bovenkamp M, Bruinen de Bruin Y, Delmaar C, van Engelen J, Escher S, Marquart H, Meijster T 2010. Exposure based waiving under REACH. Regul. Toxicol. Pharmacol. 58 (3): 408-420

Hollander A, Bruinen de Bruin Y, Vonk A, Vermeire T, van de Meent D 2011. Exposure based waiving in environmental risk assessment: a tiered approach. Human Ecol. Risk Assess., accepted

## Derivation of threshold values for inhalation exposure (Partners FhG, TNO)

The Threshold of Toxicological Concern concept is one constituent of the Integrated Testing Strategy for chronic toxicity developed within OSIRIS. If human exposure does not exceed the defined TTC limit values, no risk to human health is expected. TTC values are used for the risk assessment of substances when there are no toxicological data available or testing is not possible for technical reasons. TTC have already been used successfully to regulate e.g. food contaminants and flavourings substances.

Based on the substances' structural properties, the TTC concept distinguishes three substance classes and their corresponding thresholds by means of the Cramer decision tree. Cramer classes 1 and 2 include substances whose structure suggests low/moderate toxicity, while Cramer class 3 contains all substances with predominantly reactive structural groups which are



PEC-distribution of DBP in surface water.



	Number of compounds	TTC ( $\mu\text{g}/\text{person}/\text{day}$ ) for Cramer class #		Inhalation TTCs derived for consumers based on the RepDose database. # exposure: 24h/day and 7days/week
		1	3	
All compounds	203	71	4	
Non-genotoxic	136	180	4	

expected to cause toxic effects. The Cramer decision tree is based on theoretical considerations and was already developed in 1978 to assess systemic toxicity. In 1996, Munro made use of the Cramer classes to derive TTC values for oral exposure. To this end, he developed a database commonly referred to as Munro database, which contains the NOEL and LOEL values (No Observed Effect Level and Lowest Observed Effect Level) of over 600 substances from mostly subchronic and chronic studies in rats, mice, hamsters, and rabbits. The threshold values he derived were 1800  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 1, 540  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 2, and 90  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 3.

Inhalation is an important route of exposure to chemicals at the workplace. In this investigation it has been evaluated whether and to what extent the TTC concept is suitable for deriving threshold values for substances taken up by inhalation. TTC values for inhalation exposure to non-genotoxic substances were derived by using the FhG database RepDose ([www.Fraunhofer-RepDose.de](http://www.Fraunhofer-RepDose.de)).

In the RepDose database, 203 industrial chemicals were identified that have already been tested in repeated-dose inhalation studies. Threshold values were derived by using an analogous method to that developed by Munro, and these were 4  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 1 and 71  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 3. No value could be derived for Cramer class 2, as this class included only a very small number of substances (4%).

The derived thresholds for inhalation exposure are significantly lower than the TTC values for oral exposure. It has been demonstrated that one reason for the observed difference between inhalation and oral thresholds is the sensitivity of the respiratory tract to local effects. Local effects in the respiratory tract are frequently observed, even at low exposure concentrations, and thus determine the NOEC.

In a next step, all substances with structural alerts for genotoxicity were excluded, since genotoxic substances are regulated by a specific TTC value of 0.15  $\mu\text{g}/\text{person}/\text{day}$ . This resulted in the following TTC values for non-genotoxic substances: 180  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 1 and 4  $\mu\text{g}/\text{person}/\text{day}$  for Cramer class 3.

Under the European Regulation REACH, risk assessments of thousands of chemicals will be required within the next few years. Together with the oral TTC values already described in the literature, the inhalation thresholds derived in this work represent a useful and transparent method allowing to avoid animal testing if the exposure is below the substance-specific threshold value. By taking into account route-specific differences, it will be possible to further improve the TTC concept and thus also the corresponding thresholds.

Escher SE, Tluczkiewicz I, Batke M, Bitsch A, Melber C, Kroese ED, Buist HE, Mangelsdorf I 2010. Evaluation of inhalation TTC values with the database RepDose. *Reg. Toxicol. and Pharmacol.* 58: 259-274

## Database on (eco)toxicity data available from Russian language sources (Partner NICPB)

Toxicity data are collated for the development of (quantitative) structure-activity relationships and Integrated Testing Strategies. A novel aspect of data collection has been the searching of literature published in Russian in the former Soviet Union and Russia. The search performed in Estonian and Russian libraries and on websites covered books, journal articles, electronic databases and PhD dissertations.

The web-database E-SovTox (<http://kbfi-databases.eu/database>) including eco- and toxicological data has been created using PHP and SQL. The database contains not only numerical data but also literature references and abstracts in two languages (Russian and English) as well as original documents as pdf files. The search engine can be used both with Roman and Cyrillic characters. In the database, each compound is presented on a separate page including:

- General information (CAS no, molecular weight, synonyms, common usage)
- Direct access to extended search engines
- Mammalian toxicological / ecotoxicological data
- Comments, references etc.

Toxicological data originate mainly from the Russian journal „Industrial Hygiene and Occupational Diseases“ (Гигиена труда и профессиональные заболевания), ecotoxicological data from the Russian journals “Hydrobiological Journal” (Гидробиологический журнал) and “Inland Water Biology” (Биология внутренних вод).

Altogether ~490 articles and ~650 substances with toxicological data and 44 ecotoxicological papers and 42 compounds are currently included in the database.

Sihtmäe M, Dubourguier HC, Kahru A 2009. Toxicological information on chemicals published in the Russian language: contribution to REACH and 3Rs. *Toxicology* 262 (1): 27-37

Sihtmäe M, Blinova I, Aruoja V, Dubourguier H-C, Legrand N, Kahru A 2010. E-SovTox: An online database of the main publicly-available sources of toxicity data concerning REACH-relevant chemicals published in the Russian language. *ATLA* 38 (4): 297-301

## Across-species relationships and toxicogenomic approaches (Partners UA, UNEXE, UB, VU, NICPB, KWR)

Mechanistic information is becoming a requirement for successful modelling and risk assessment and toxicogenomic techniques are promising techniques to elucidate the mode of action (MOA) of toxicants. The main objectives of research in this workpackage are to identify similarities and dissimilarities in response and MOA between toxicological model organisms after exposure to the test chemicals, to assess the power of transcriptomics in terms of unravelling MOAs and to assess the applicability of alternative assays. This will reduce the uncertainty due to differences in species susceptibility, and will allow to refine the risk assessment process. Furthermore, transcriptomics profiles provide insight in differences in MOA among similar and dissimilar compounds.

The acute toxicity of four case study compounds (aniline, 4-chloroaniline, 3,5-dichloroaniline and 2,3,4-trichloroaniline) was tested in an interspecies context using different alternative cell and organism based assays: neutral red cytotoxicity assay with the EPC cell line of *Cyprinus carpio*, luminescent bacteria test with *Aliivibrio fischeri* at 15° C and 20°C, bacterial growth inhibition test with *E. coli*, algae growth inhibition test with *Chlamydomonas reinhardtii*, algae growth inhibition test with *Pseudokirchneriella subcapitata*, immobilisation test with *Daphnia magna*, reproduction toxicity test with *Folsomia candida* and fish embryo toxicity tests with *Danio rerio* for 48h. The obtained results indicate that the toxicity of the anilines depends on the degree of chlorosubstitution, i.e. structure of the chemicals, and also varies among the different test species. The general hypothesis of increasing toxicity with increasing chlorosubstitution was observed in all test systems except for *Daphnia magna* and *Folsomia candida* where an opposite response was seen.

The use of mode of action-based QSARs requires fundamental knowledge on the chemical as well as on the biological mechanisms of the compounds. The emphasis in MOA determinations, however, is too often based on chemical descriptors while biological descriptors are underrepresented. Consequently this can lead to MOA misclassification and wrong toxicity predictions. Biology-based alternatives for MOA prediction need to be considered.

Toxicogenomics is a promising technique to assess MOAs of

chemicals. Different chemicals with different MOAs potentially give rise to different molecular fingerprints. The starting hypothesis is that compounds associated with similar mechanisms of toxicity yield similar gene expression profiles, which are distinct from profiles generated by other classes of chemicals. This means that chemicals from the same chemical category are likely to share a similar molecular fingerprint. It is hypothesised that by identifying “key genes or key clusters of genes”, chemicals can be categorised.

The biological MOA of the four test compounds were tested. In order to test the power of transcriptomics, (dis)similarities in toxic biological MOAs were identified. Gene expression analyses were performed in rainbow trout cell line, RTL-W1, *Danio rerio* embryo, *Folsomia candida*, *E. coli*, *Chlamydomonas reinhardtii* and *Daphnia magna*. In addition, the Ames II mutagenicity test has been carried out for species comparison. The gene expression data were used to support and explain toxicity effects that were observed at higher levels of biological organisation. These data will be used to assess the potential of biological descriptors in compound classification and class prediction.

Dom N, Nobels I, Knapen D, Blust R (2011). Bacterial gene profiling assay applied as an alternative method for mode of action classification: pilot study using chlorinated anilines. *Environ. Toxicol. Chem.* 30: Vol 30 (5): 1059-1068

## Data quality assessment for in silico methods (Partners AL, RIVM, IRFMN, ISS, LJMU, FhG, URV, WUR, IVZRS, TNO, UB, MERCK)

A joint action of 21 contributors from 12 OSIRIS partner institutions has presented common grounds for data quality within (and beyond) OSIRIS from different (inter)disciplinary perspectives. The project report has been published as a chapter of the book “In Silico Toxicology. Principles and Applications” edited by M. Cronin and J. Madden (LJMU).

Integrated Testing Strategies (ITS) aim to use and combine existing data for human and environmental risk assessment purposes while minimising the need for new testing. REACH has advocated a Weight of Evidence (WoE) approach to decide whether information is adequate to draw a conclusion on, e.g., the toxicological properties of a substance. To determine how much a piece of information should contribute to the overall conclusion, the validity of methods needs to be assessed as well as the reliability and relevance (fit-for-purpose) of this information.

Data quality assessment needs to address multiple issues at several levels:

- Individual data quality: The variability in pieces of information depends on confounding factors in the (experimental) procedure used to generate the data.

In silico predictions must include an assessment, at the very least, of the error range of the experimental data that were used to derive the model. Variability of toxic effect data can be due to either technical (e.g., identity of test substance, deviations of test protocols, differences in exposure conditions) or inherent biological (e.g., species, strain, age and sex of test animals, seasonal influence) factors.

- Combined data quality: Less reliable data can still be adequate for risk assessment in combination with other evidence. The pooling of several studies, one or more of which may be inadequate by itself, may collectively satisfy the overall requirement for valid data.
- Context-dependent data quality: Different levels of data quality are required for different purposes. For example, read-across requires a very high confidence in each of the few data points it uses; Qualitative/quantitative structure-activity relationships (QSARs) demand increasing confidence in the experimental input data with decreasing number of substances in the training/test set; and evidence-based toxicology (EbT) may cope with mixed variability.

Variability is an obvious first measure of data quality, but what is actually required is an understanding of the degree of (un) certainty. The ultimate objective of data quality assessment is to identify, reduce and communicate uncertainty in decisions based on data.

The acceptable uncertainty of data (measured or generated in silico) in the regulatory context depends on the outcome of the hazard identification and risk assessment and can also be weighted for the „cost“ of errors.

Data quality assessment is a complex and time-consuming task, but exceptionally important as models derived from poor quality data will only deliver poor predictions. The OSIRIS report supports and encourages the crucial process of data quality assessment with a focus on specific needs in in silico toxicology by providing

- Common terms and definitions,
- Background information on formal data quality scoring schemes,
- Description of chemical and biological factors of data variability,

Nendza M, Aldenberg T, Benfenati E, Benigni R, Cronin M, Escher S, Fernandez A, Gabbert S, Giralt F, Hewitt M, Hrovat M, Jeram S, Kroese D, Madden J, Mangelsdorf I, Rallo R, Roncagliani A, Rorije E, Segner H, Simon-Hettich B, Vermeire T 2010. Data quality assessment for in silico methods: A survey of approaches and needs.

In: Cronin M, Madden J (eds): In Silico Toxicology. Principles and Applications, RSC Publishing, Cambridge, UK



- Checklist approaches to data quality assessment,
- Data quality considerations for physico-chemical data, calculated QSAR descriptors as well as environmental and human toxicity,
- Data quality needs in data integration and socio-economic evaluations of ITS by means of cost-effectiveness analysis.

## Chemical domain of QSAR models from atom-centred fragments (Partner UFZ)

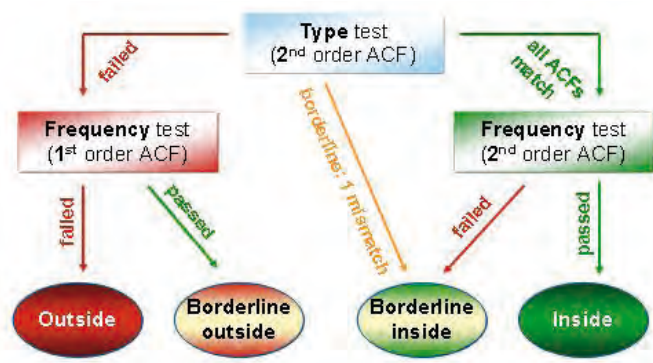
The knowledge of the applicability domain is crucial to correctly apply qualitative or quantitative structure-activity relationship (QSAR) models, as pointed out in the OECD guidelines for QSAR model validation. The applicability domain comprises the chemical space, the biological domain, etc. If a test compound is outside the chemical domain, the model reliability decreases, and the probability to obtain wrong estimations increases.

Regarding the chemical space, several aspects have to be taken into account, e.g. the physico-chemical domain, structural features, mechanistic aspects and also the metabolic domain in case that biotransformation is involved. Key properties affecting the bioavailability such as water solubility and partition coefficients of the compound of interest, as well as all descriptors applied, should not be outside the range of the corresponding properties in the model training set.

To address the structural aspects of the chemical domain, atom types, structural complexity, and polarity may be considered. So far there is no generally accepted approach to define the chemical space of QSAR models. We have suggested a new technique to characterise the structural domain of compound sets based on the atom-centred fragments (ACF) concept. Molecules are subdivided into structural fragments consisting of a central atom and bonding neighbours. An ACF is defined through the atom type and the number and type of bound neighbours, and the respective bond types. The usefulness of this approach to locally correct estimation errors, to select appropriate models, and for a semi-quantitative kNN (*k* nearest neighbours) read-across model has already been shown by our group.

While some ACF applications with regard to the model domain already exist in the literature, there has been no detailed guidance and in particular no optimised procedure available to achieve this yet. Employing several data sets for continuous and categorical models, a new approach has been developed to characterise the chemical domain based on ACFs with path lengths of one and two bond lengths, i.e., for each atom considering its next neighbour atoms and their neighbours. A criterion is obtained to decide whether a prediction compound is within the chemical space of a QSAR model with respect to the training set. To further define the model space, four categories were determined: inside, borderline inside, borderline outside, and outside and illustrated with examples.





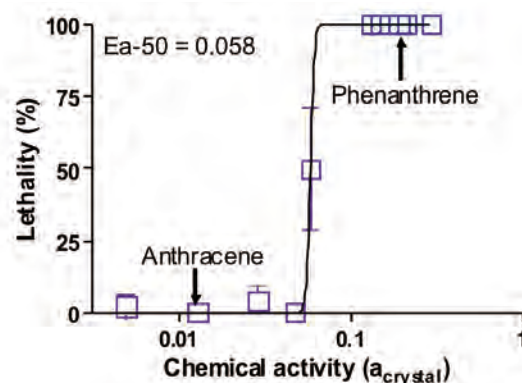
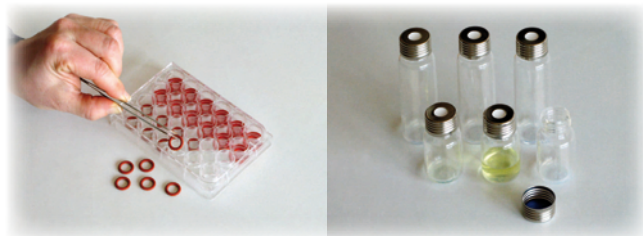
This new technique can be applied to any compound-related model developed or data set provided. In particular, all QSAR models developed in our group will contain respective checks. The method is already available in the OSIRIS edition of the ChemProp software.

Kühne R, Ebert R-U, Schüürmann G 2009. Chemical domain of QSAR models from atom-centered fragments. *J. Chem. Inf. Model.* 49: 2660-2669

## Passive dosing to control hydrophobic organic substance concentrations in toxicity tests (Partner AU)

Many toxicity test systems are prone to evaporative and sorptive losses of hydrophobic organic compounds (HOCs), compromising the validity and applicability of the test results. Passive dosing by equilibrium partitioning from a polymer is a strategy to establish and maintain constant and well defined HOC test exposure levels. The HOC is provided by partitioning from a dominating polymeric phase, rather than by adding it via a solvent extract. Advantages include controlled and constant freely dissolved concentrations (or chemical activity), and that organisms are not co-exposed to solvents.

Silicone has been selected as the optimal polymer, and its compatibility with different test organisms tested and confirmed. HOC loading into the polymer and release kinetics into the test medium have been systematically investigated for various passive dosing formats. Equilibrium freely dissolved concentrations are rapidly reached, and maintained for the test duration. Passive dosing has been applied successfully in a range of invertebrate and in vitro toxicity assays. As an example, the limit toxicity (= at solubility) of 10 polycyclic aro-



Lethality of the springtail *F. candida* after 7 days exposure to 10 PAHs plotted against the chemical activity of the PAHs. Lethalities (mean  $\pm$  stdev) of all PAHs were fitted to one uphill function (Mayer P and Holmstrup M 2008. *Environ. Sci. and Technol.* 42: 7516-7521).

matic hydrocarbon (PAH) compounds to the springtail *Folsomia candida* has been determined using the passive dosing vials.

Smith KEC, Oostingh GJ, Mayer P 2010. Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during in vitro toxicity tests. *Chem. Res. Toxicol.* 23: 55-65

Smith KEC, Dom N, Blust R, Mayer P 2010. Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing. *Aquat. Toxicol.* 98 (1): 15-24

## Sources of variability of in vivo ecotoxicity data (Partners IVZRS, UB)

The variability of fish acute lethality values as listed in commonly used databases was investigated for 44 chemical substances (4 654 records) extracted from the US EPA ECOTOX database. Significant variability in test results going up to several orders of magnitude for one and the same substance was observed.

In an attempt to systematically explore potential sources of the data variability, the influence of test species, life stages and test conditions (temperature, pH, water hardness) was analysed. Major limitations in this analysis were pronounced gaps in the recording of test conditions. Amongst the 4 654 extracted reports 66.5% of data were without information on fish life stage. Mean temperature, water hardness and pH were not defined in 19.6%, 48.2% and 41.2% respectively. For 75.4% of reports chemical purity was not recorded.

As conclusion from the results, it is suggested on the one hand to optimise acute toxicity test protocols in order to minimise variations due to variable testing procedures, on the other hand, to rigorously control data quality before they are entered into databases.

With respect to in vivo toxicity testing, a first recommendation from this study is to reduce the number of accepted fish species, life stages and test protocols. This could clearly reduce data variability. For instance, initial data evaluation could always be focused first on rainbow trout, as one of the most sensitive

species and the species with comprehensive data already available, while additional species would only be considered if there is a lack of trout data or for specific purposes. A similar proposal is contained in the guidance document on aquatic ecotoxicity developed for the purpose of pesticide risk assessment which requires the acute toxicity test on *Oncorhynchus mykiss* as mandatory and an additional test on warm water fish. The restriction to one key species would also enable a more narrow setting of test conditions, that would further reduce variability.

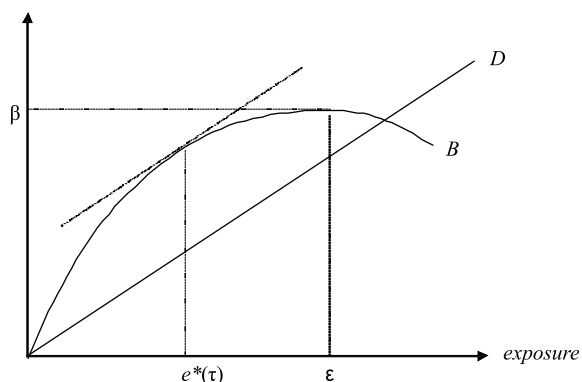
A second recommendation is a more stringent reporting of test conditions and parameters, together with a more strict selection of data entries for databases. A more detailed reporting of test procedures and conditions would be important to understand why results from individual tests are different. Such effort would greatly reduce data variability and thereby would enhance the utility of databases used in the development of non-testing and non-animal testing methods. Moreover, the need for repetition of studies and thus the number of fish used for testing would be significantly reduced.

Hrovat M, Segner H, Jeram S 2009. Variability of in vivo fish acute toxicity data. Regul. Toxicol. Pharmacol. 54 (3): 294–300

## A decision analytic value-of-information approach for test prioritisation and ITS optimisation (Partner WUR)

Risk management of chemicals requires information about their adverse effects such as, for example, toxicity and persistence. Testing of chemicals allows for improving the information base for regulatory decision-making on chemicals' production and use. Testing a large number of chemicals with limited time and resources forces a prioritisation of testing, i.e. a rank order of chemicals such that higher ranked substances are to be tested earlier than lower ranked substances. A decision model for the prioritisation of chemicals for testing has been developed: The model adopts a value-of-information (VOI) approach

benefits  
damage costs



describing the expected welfare gains from regulatory actions that respond to test information revealing chemicals' level of toxicity and persistence. Hence, the VOI model suggested can be applied to both human health and environmental endpoints. The expected welfare gain of improved regulation is the value of information.

It is assumed that exposure ( $e$ ), is the variable that the regulator can control. Hence, safety measures are modelled as a reduction of exposure to toxic or potentially toxic chemicals. As shown in the figure, in the absence of regulations the maximum benefits that could be obtained from the use of a substance are  $\beta$  (i.e. where marginal benefits equal zero). Thus,  $e$  denotes the unregulated level of exposure when damages are externalities and are not accounted for.

Given safety measures  $e$ , the substance should only be produced if benefits ( $B$ ) exceed the damage ( $D$ ) from the use of a substance, i.e. if  $B(e) \geq D(e, \tau)$  with  $\tau$  denoting a substance's toxicity potential. The optimal regulation, i.e. the optimal expected level of exposure  $e^* \tau$ , is where marginal benefits equal marginal damage, which is given by the solution to  $\max_e [V = B(e) - e E(\tau)]$  (with  $E$  denoting the expectations operator).

The expected VOI is the expected gain when using the substance if optimally regulated with additional information from testing, instead of using the substance regulated under uncertainty. A test should be performed if and only if its VOI exceeds its costs.

If the VOI from testing net of costs of testing differs between two substances, then the test with the higher net value of information should be performed first. In this way all substances can be prioritised with regard to testing. Hence, ultimately, the VOI is driven by the effectiveness of regulatory actions which determine human and environmental exposure to chemicals.

The analysis generally supports the use of the prioritisation criteria adopted in REACH. Chemicals known as highly toxic or highly persistent should be tested first because the required testing offers a higher VOI than testing substances without evidence of either toxicity or persistence. This effect is particularly strong if persistence is known, which supports the early deadline for substances already classified as PBT or vPvB as adopted in REACH. In addition, the decision-model accounts for other relevant prioritisation criteria that REACH ignores. For example, test prioritisation depends on the VOI net of testing costs. Testing costs may differ substantially between substances, for example when prior information differs. Hence, a regulator who sets the rules for prioritisation cannot disregard testing costs but must balance the welfare gains from improved regulation against the costs. This holds in particular if testing costs do not only comprise direct monetary costs but also include animal welfare loss. Accounting for testing costs may trigger the development of more efficient testing strategies such as, for example, ITS.

Gabbert S, Weikard H-P 2010. A theory of chemicals testing and regulation. Natural Resources Forum 34 (2): 155-164

Gabbert S, Van Ierland EC 2010. Cost-effectiveness analysis of chemical testing for decision-support: How to include animal welfare? Human and Ecological Risk Assessment 16 (3): 603-620

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