Theme 6
Modelling

Chairs:
Bernward Garthoff (Germany)
Richard Phillips (USA)

Session 6.1
QSAR acceptance and implementation

Lecture

The use of *in silico* technologies to predict toxicity and fate: Implementation and acceptance

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Recent years have seen a growing emphasis towards the use of computational methods, such as Quantitative Structure-Activity Relationships (QSARs), to predict toxicity and fate. Interest has been highlighted by their possible use in product development and regulatory applications, not least the Cosmetics Directive and REACH within Europe. The time for rhetoric is at an end, and there is now a need towards implementing these techniques. This presentation will discuss the possibilities, practicalities and limitations of the use of *in silico* technologies, specifically in a regulatory setting. In particular, the vision for the use of *in silico* techniques goes beyond traditional QSARs and expert system. There is the potential for a shift in the toxicological paradigm towards intelligent and integrated testing strategies that incorporate decision support systems. These could bring together existing data, read-across as well as predicted values. To assist the vision becoming reality, the practicalities of implementation into a regulatory setting must be borne in mind. These will include whether we accept valid, as opposed to validated, models, and how integrated strategies may be assessed and used.

Poster

A category approach for reproductive effects of phthalates

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In regulatory toxicology, the experimental assessment of reproductive toxicity is most costly, complex and time-consuming and requires the highest number of test animals. Grouping of chemicals into categories is one of the approaches that can be used to reduce the number of animal tests in safety and risk assessments of chemicals. This is expected to play an important role also in the future new EU chemical policy called REACH. The application of the category approach for reproductive toxicity endpoints was investigated, using phthalates as an example. A group of 10 ortho phthalate esters was selected to categorise the phthalates that produce severe reproductive effects in experimental animals and those that do not. The differences in physicochemical properties, absorption rates and metabolism between the phthalates investigated could not explain the difference in their reproductive toxicity. It appeared that phthalates with side chain length from C4 to C6 can produce similar reproductive effects in experimental animals. The anti-androgenic effects observed in post-organogenesis in *in vivo* studies seem to be the most crucial. From this investigation it is expected that phthalates included in the tight boundaries of this category would all show these anti-androgenic effects. Further testing might not be needed for phthalates within these boundaries. For phthalates outside the boundaries the reproductive potential remains unclear.

Lecture

Validation of a set of physical limit rules for no irritation or corrosion

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(Q)SARs will need to be used for assessing chemicals to limit animal testing in the framework of REACH and cosmetic directive. Regulatory use of these methods will increase when the tools are officially validated. We present the validation of the existing (Q)SAR rule base of the BfR (former BgVV), based on new chemicals for predicting the skin irritation and corrosion potential of chemicals. This rule base predicts non-irritation and non-corrosion by means of physical-chemical limit values and special classes of empirical formulas, such as CHal and CN. Within this project we will re-evaluate the rules, taking into account the OECD principles on (Q)SAR validation. We will also externally validate the rule base using 200 new chemicals that were not used for developing the model. Since these rules address the absence of irritation/corrosion, the number of false negatives, if any, will be reported. The number of chemicals of the validation set that predicts the absence of corrosion/irritation will be shown. This number also presents the number of tests that can be omitted in case this rule base is applied. The question as to whether this external training set of 200 chemicals fully covers the applicability domain of the rule base will be presented. This work is directly related to the presentation on the SICRETool that is a tiered approach assessing the skin irritation/corrosion potential without *in vivo* testing of Walker et al. as it validates the first step in this tiered approach.

Lecture

Applicability domain of a QSAR assessed in parameter and structural space

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Applicability domain (AD) of a QSAR has been so far estimated on the basis of model descriptors' space coverage by the training set. Different numerical approaches to estimate this subspace yield different ADs. Particular choice depends on training set data distribution and dimensionality of the model (i.e. number of descriptors). In general, interpolative predictive accuracy within descriptor space is on average greater than extrapolative predictive accuracy. That however, is only true on average, i.e. there are many individual compounds with small error outside of the descriptors space coverage, as well as individual compounds with large error inside the domain. By identification of descriptor coverage, we make only a partial step towards defining model's AD. There is always a possibility that the model is missing a descriptor needed to correctly predict a queried chemical's activity. Then, despite the chemical being in the parameter domain, the chemical's activity is predicted with error. To address this problem and refine AD estimation we propose to add a global structural similarity test to ensure that the structural features in a new test compound are covered in the original training set of chemicals. These two conditions: 1) being in the model's parameter space coverage and 2) structural similarity to the training set are complementary. Therefore, in order to describe the domain robustly, the full training set comprising both structures and descriptor set is required. We will provide case study of mutagenicity QSAR by Debnath et al. (1998) AD estimation as example of the proposed approach.

Poster

Introduction to the Artificial Neural Networks (ANNs) and their applications in QSAR studies

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QSAR studies rely upon statistics and other techniques to derive mathematical models which relate the biological activity of a series of compounds to one or more properties of the molecules. These properties, or descriptors, may be derived from numerous sources including refractive index, octanol/water partition coefficient or spectral data. Many methods were used for mathematical modelling in QSAR studies with few or many limitations and assumptions e.g. regression analysis in QSAR model building with assuming linear relationship between the biological activity and one or more descriptors. Artificial Neural Network modelling is one of such affords originated from a field called Artificial Intelligence (AI). Artificial Neural Networks (ANNs) are the mathematical algorithms generated by computer that approach the functionality of small neural clusters in a very fundamental manner. For their application in QSAR studies, the individual network is built and trained with the source data and actual biological activity. The trained network is useful for predicting the biological activity of presented new chemical compounds. In this paper, the author is going to introduce the Artificial Neural Network technology, the basic concepts and working of Artificial Neural Networks and their possible applications in QSAR studies.

Poster

Consensus classification of chemicals according to the mechanism of toxic action to fish

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Quantitative Structure-Activity Relationships (QSARs) rely on the paradigm that chemicals belonging to the same or similar chemical classes behave in a similar manner. In the field of aquatic toxicology, it is widely agreed that the QSARs are valid for prediction within the same applicability domain, i.e. for the same mechanism of toxic action (MOA). The aim of this study was to perform consensus classification according to MOA of 177 chemicals taken from the OECD Screening Information Data Set (SIDS) for high production volume chemicals. For this purpose four classification schemes were compared. The first scheme was applied in-house and used to classify chemicals into seventeen MOA. The second one was done by an expert and included a similar number of mechanisms. The third one was performed according to the rules implemented in ASTER (Assessment Tools for the Evaluation of Risk, U.S. EPA – Duluth MN). A consensus classification based on the majority principle was achieved comprising nine MOA. The consensus MOA of the 177 chemicals were then compared with the classifications obtained by applying the scheme of Verhaar et al., 1992, *Chemosphere 25*, 471-491. As a result, 75 chemicals were classified as non-polar narcotics (NPN) and 12 as polar narcotics (PN). Their acute toxicity to fish can be predicted confidently by the NPN and PN models, including those recommended by the Technical Guidance Document of the European Commission. The remaining 90 chemicals were classified as reactive and for prediction of their toxicity we suggest the use of MOA-specific QSAR models.

Poster

The use of similarity measures in defining the applicability domain of skin sensitisation SARs

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In the (Q)SAR field, the applicability domain (AD) is widely understood to express the scope and limitations of a model, i.e. the range of chemical structures for which the model is considered to be applicable. For QSAR models, the parameter space is typically represented by defined ranges of physicochemical descriptors. For SAR models in the form of structural alerts, the parameter space is typically represented by the structural feature that defines the presence of a hazard.

One potential approach to prevent the inappropriate application of a (Q)SAR model involves the use of similarity measures to compare a new query chemical with those present in the training set. Such a similarity measure should ideally reflect the mechanistic basis for the (Q)SAR, although the use of structural analogy alone may be appropriate in situations where the mechanism is unclear.

This work explores the use of similarity measures for a set of structural rules and investigates their utility in the validation of (Q)SARs. Examples are based on structural rules for skin sensitisation.

Lecture

SAR meets LLNA – Structure Activity Relationship triggers the Local Lymph Node Assay for testing the skin sensitisation potential

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One of the fundamental toxicological issues for chemical substances is their skin sensitisation potential. Therefore, regulators require proper skin sensitisation tests both with guinea pigs or the Local Lymph Node Assay (LLNA) with mice for hazard identification, and proper risk assessment when chemicals are intended for dermal contact, like cosmetics.

Driven by the interest to follow the 3R principles: refinement, reduction and replacement of animal tests, there is an urgent need to optimise currently accepted test methods. Chemical specific parameters have been calculated by a newly developed computer program and result in a Structure Activity Relationship (SAR), predictive for the estimation of LLNA test results. Such theoretic estimation of sensitisation potential is useful to focus the animal tests on the most promising chemicals.

Based on the hypothesis that the skin sensitisation potential is related to specific chemical structures, the SAR has been calculated and in parallel, officially required LLNA sensitisation tests have been conducted. Out of about 100 test substances, chemical groups were defined according to their complex physicochemical parameters in respect to these test results. Such SAR information was found to be an added value for the screening of new chemical substances as well as for optimising individual LLNA test protocols.

The results demonstrate that SAR data are scientifically helpful to understand the interaction of chemicals with the immune system and prospectively helps to reduce the need of animal tests.

Lecture

Roles for QSAR in risk assessment

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Early pioneers in QSAR development believed in the premise that laboratory experiments should not be performed without a firm expectation of the results before going into the laboratory. The value of QSARs in risk assessment will be to provide us with those expectations for a wide variety of exposure and hazard assessment endpoints before the decision to require specific testing is made. Most of the current QSAR models are limited only by the lack of designed databases; however, as they evolve, QSAR models for most endpoints will undoubtedly be used to provide us with test expectations for thousands of untested chemicals. In so doing, QSAR will complement the 3Rs with a powerful new tool to minimise animal testing which is not likely to influence regulatory decisions. If it is true that 95% industrial chemicals have lower probability to be classified as an EDC than n-butyl aniline, avoiding testing on those chemicals can be achieved by QSAR screening. With the recent development of computer simulators of metabolic activation (e.g. the virtual liver), improved QSAR models for skin sensitisation, respiratory irritation and genotoxicity will follow quickly. Finally, the integration of QSAR models with *in vitro* methods holds great promise in the prudent use and interpretation of our testing and assessment resources.

Lecture

The assessment of the skin irritation/corrosion potential without *in vivo* testing

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This presentation shows the possibility of assessing the skin irritation/corrosion endpoint without or limited further animal testing using a tiered approach called SICRET (Skin Irritation Corrosion Rules Estimation Tool). The proposed mechanism behind irritation is that an organic chemical first needs to penetrate the skin before the reactivity of the chemical can cause cytotoxicity leading to irritation or corrosion. This mechanism is used as a starting point in the tiered approach. As a first step physicochemical limit values were derived from a database of circa 1300 chemicals. For chemicals outside these limit values no irritation/corrosion is expected and therefore need not be classified. The validation of these physicochemical rules will be presented by Hulzebos et al.. Chemicals that fall within these limit values follow the second path in the tiered approach. These chemicals need to be checked for irritation or corrosion alerts and if present they can be classified accordingly. For chemicals with dermal absorption potential and no structural alerts prevalidated *in vitro* testing is proposed. If also this result shows no irritation/corrosion we propose to delete the *in vivo* test for confirmation requested in the OECD guideline. Though this endpoint and this strategy might not score high on the number of animal saving it scores on animal welfare. In summary, SICRET is a "tiered approach" that uses physicochemical property limits, structural alerts and *in vitro* tests to classify chemicals that cause skin irritation or skin corrosion without further animal testing.