Preface

Exemplification of the Implementation of Alternatives to Experimental Testing in Chemical Risk Assessment — Case Studies from the CADASTER Project

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REACH and the Need for Alternative Testing

The EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) system (hereafter called REACH) requires the demonstration of the safe manufacture of chemicals and their safe use throughout the supply chain.[1] REACH is based on the precautionary principle, but aims to achieve a proper balance between societal, economic and environmental objectives. Both new and existing chemicals will be evaluated within REACH, on the one hand aiming to speed up the slow process of risk assessment and risk management of existing substances, whilst on the other hand attempting to efficiently use the scarce and scattered information available on the majority of new and existing substances. REACH thus aims to close huge gaps of knowledge on physicochemical properties and adverse effects for large numbers of chemicals. In addition, REACH aims to reduce animal testing by the optimised use of qualitative and quantitative information on related compounds.

The REACH proposals advocate the use of non-animal testing methods, but guidance is needed on how these methods should be used. As an example, the REACH system requires that non-animal methods should be used for the majority of tests for chemicals in the 1–10 tonnes per annum production band, even though such methods are not yet available for most of the endpoints relevant at this tonnage. In an attempt to resolve the issue of lack of guidance, the European Commission made the following suggestions on how *Reduction*, *Refinement* and *Replacement* strategies could be applied to animal use in the REACH system:

1. Encouragement of the use of validated *in silico* techniques such as (Q)SAR models.

2. Encouragement of the development of new *in vitro* test methods.

3. Minimisation of the actual numbers of animals used in the required tests, and the replacement of animal tests wherever possible by alternative methods.

4. Formation of Substance Information Exchange Forums (SIEFs) for the obligatory provision of data and cost sharing.

5. Requirement of official sanctioning of proposals for tests for compounds with production volumes above 100 tonnes per annum, to minimise animal testing.

Operational procedures that guide a transparent and scientifically sound evaluation of chemical substances in a risk-driven, context-specific and substance-tailored manner are to be developed, tested, and disseminated. Various alternatives are available to supplement existing data, or even to substitute for toxicity data which are lacking, as advocated within REACH in order to reduce unnecessary animal testing. In line with the paradigm shift that has taken place when establishing REACH — of performing risk management instead of risk assessment[2,3] — the concept of Intelligent (or Integrated) Testing Strategies (ITS) was developed to optimise the integration of the available experimental data and alternative means of assessing adverse effects, whilst adhering to one of the main objectives of REACH of minimising the use of test animals.

Intelligent (or Integrated) Testing Strategies

Intelligent, or Integrated, Testing Strategies are the most efficient way to obtain the necessary

information to carry out hazard and risk assessments of large numbers of chemicals, while reducing costs to industry and minimising animal testing. ITS are integrated approaches comprising multiple elements aimed at speeding up the risk assessment process, while reducing costs and animal testing.[2] Within ITS, all alternatives to experimental testing are integrated, as is schematically exemplified in Figure 1.

As indicated in Figure 1, experimental testing within ITS is carried out only as a last resort, i.e. when no information at all, or when no reliable information, can be obtained by means of any of the following alternatives:

— Quantitative structure–activity relationships or quantitative property–activity relationships (QSARs or QSPRs);

— Read-across, commonly performed by interpolating information on related compounds;

— *In vitro* testing;

— Exposure-based waiving, providing evidence that biota are not exposed to the chemical of interest, or only at concentrations well below the No Observed Effect level.

The implementation of ITS in hazard assessment boils down to what is called the Three Rs strategy of replacement, reduction and refinement of toxicity testing. Initially, though, a seven-R strategy was advocated, including the initial Three Rs:

1. *Risk-based strategy:* Focus on risks (include exposure);

2. *Repetitive:* A tiered approach should be applied, going from simple, to refined or comprehensive (if necessary), in order to quickly assess chemicals of low concern and to avoid animal testing;

3. *Relatives:* The focus should be on families or categories of chemicals (a group-wise approach) by using read-across, QSARs and exposure categories (i.e. a move away from the chemical-by-chemical approach);
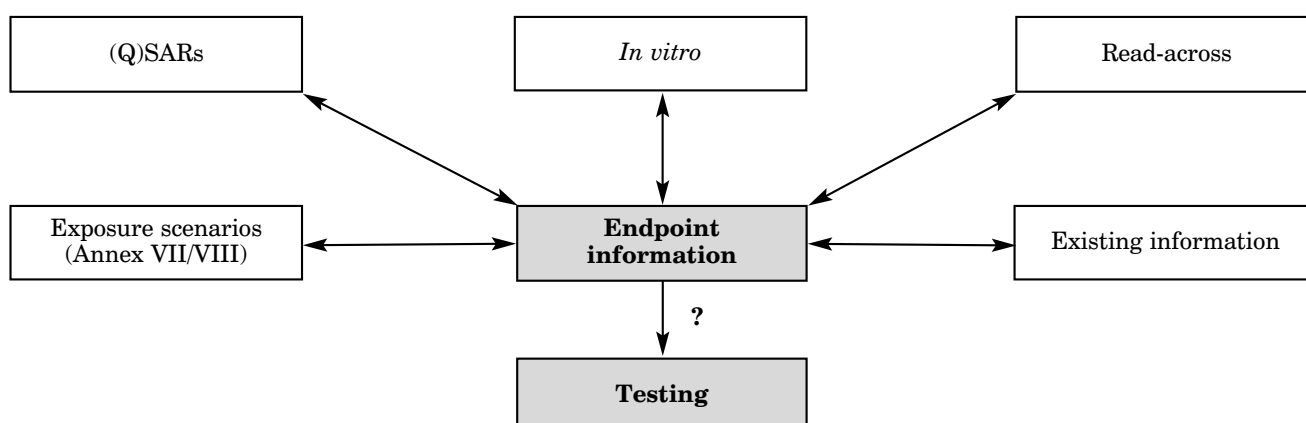
4. *Restriction (waiving) of testing:* To be implemented, where possible, and *in vivo* testing to be carried out where needed, in order to prevent damage to human health and/or the environment;

5. *Replacement:* Substitution of the need for tests on conscious living vertebrates;

6. *Refinement:* To decrease the suffering and distress of test animals; and

7. *Reduction:* To reduce the numbers of animals used in testing.

Concerted action and intensive efforts are needed to operationalise all possible alternatives into a workable, consensually acceptable, and scientifically sound strategy for the hazard and risk assessment of large numbers of chemicals. The production of guidance and (web-based) tools is essential in this respect. So far, the use of non-testing methods in the European regulatory context is quite limited and fragmented. The reasons for this include the lack of distinct application criteria and guidance, and the fact that uncertainty has not been addressed rigorously. Industry is primarily made responsible for carrying out the risk assessments, and practical guidance is therefore needed on how to apply the elements of newly-derived testing strategies in a consistent manner.

The CADASTER Project

It is within this context that the CADASTER (**CA**se Studies on the **D**evelopment and **A**pplication of *In Silico* **T**echniques for **E**nvironmental Hazard and **R**isk Assessment) project was designed. The aim of

Figure 1: The Elements of an Integrated Testing Strategy

the CADASTER project is to provide practical guidance on integrated hazard and risk assessment procedures by exemplifying hazard and risk assessments for chemicals belonging to four specific compound classes, by integrating the various tools that are made available within the project for each of the four compound classes. The tools and the underlying data and models are made available via the project website (http://www.cadaster.eu), as an online and stand-alone tool for the development, publication and use of QSAR models for REACH. The CADASTER tools predict physicochemical properties and toxicities for four analysed classes. The predictions provided are compatible with the Organisation for Economic Co-operation and Development (OECD) QSAR Application Toolbox and the EPI Suite™ Toolbox developed by the EPA's Office of Pollution Prevention Toxics and the Syracuse Research Corporation (SRC). Operational procedures were developed that explicitly take account of variability and uncertainty in data and in models. The objectives of CADASTER are in line with the basic idea of REACH — to obtain the information needed for carrying out hazard and risk assessments of large numbers of substances by integrating multiple methods and approaches with the aim of minimising testing, costs and time.

CADASTER facilitates the selection of the relevant fate and effect parameters, as it supplements the existing database on fate and effect properties of the following compound classes, which were selected as the chemical classes of choice for CADASTER:

1. Polybrominated diphenyl ethers (PBDEs): These are typically a class of hydrophobic chemicals, some of which have been used as flame retardants, that pose a threat to man and the environment.

2. Perfluoroalkylated substances and their transformation products, such as perfluoroalkylated sulphonamides, alkanoic acids and sulphonates: Fluorinated compounds are typically a class of persistent, relatively hydrophilic compounds that may be toxic for man and the environment.

3. Substituted musks/fragrances: These are a heterogenic group of chemicals of varying composition. Examples include substituted benzophenones, polycyclic musks and terpene derivatives. In view of their typical use patterns, these chemicals have a common emission pattern in the environment.

4. Triazoles/benzotriazoles: These are a class of chemicals that are increasingly used as pesticides and anti-corrosives.

The main goal of the CADASTER project is to exemplify the integration of information, models and strategies for carrying out safety, hazard and risk assessments for large numbers of substances, according to the new categories of risk assessors within REACH. Real risk estimates are delivered according to the basic philosophy of REACH of minimising animal testing, costs and time. CADASTER thus shows how to increase the use of non-testing information for regulatory decision-making, whilst meeting the main challenge of quantifying and reducing the level of uncertainty.

The Second CADASTER Workshop

In this special issue of *ATLA*, a compilation of contributions from the second CADASTER Workshop in Munich (7–9 October, 2012) regarding the implementation of alternatives to experimental (*in vivo*) data in risk and hazard assessment, is provided. It includes the following topics:

— The experimental assessment of the environmental fate and effects of (benzo)triazoles, by discussing the results of generating fate and effect data that have been shown to be the essential data needed for subsequent development of predictive models.[4]

— The development of suited descriptors to define the chemical space of chemically similar compounds and the applicability domains of predictive models.[5] This contribution shows that reliable methods for representative sub-sampling in terms of experimental design and risk assessment are crucial within REACH. Experimental design approaches were developed, utilising predicted properties and the 'distance to model' parameter, to estimate the benefits of certain compounds to the quality of a resulting model. A statistical evaluation of four regression data sets and one classification data set showed that the adaptive concept to iteratively refine the representation of the chemical space contributes to a more efficient and more reliable selection, in comparison to traditional approaches. The evaluation of compounds regarding the uncertainty and the correlation in prediction is shown to be beneficial, in particular with regression data sets of sufficient size, whereas the use of predicted properties to define the chemical space is shown to be beneficial for classification models.

— The evaluation of QSAR models for aquatic toxicity of (benzo)triazoles and prioritisation by consensus:[6] This contribution focuses on the 'CADASTER chemical class' of (benzo)triazoles, and shows not only how various models can be built on the basis of the same data set, but also how consensus modelling contributes to more-confident toxicity assessment.

— The application of read-across to quantify the aquatic toxicity of chemicals belonging to the het-

erogeneous class of 'CADASTER fragrance chemicals':[7] This class of compounds does not share a single specific chemical functionality, but instead shares its use pattern, i.e. all of the substances are used as fragrances. Therefore, no specific QSAR models applicable to the (whole) class of fragrances can be made available. Broadly applicable QSAR models, which are considered valid for organic chemicals in general, could be used to predict fragrance toxicity. This contribution shows how QSAR models and various types of additional information of related compounds can be integrated into read-across predictions. The predictions are integrated to derive Predicted No-Effect Concentrations (PNECs) that are used directly in risk assessment. The PNEC values derived by means of read-across are compared to PNEC values derived on the basis of experimental (*in vivo*) toxicity data.

— The justification of why the consideration of uncertainty in QSAR predictions in probabilistic hazard and risk assessment will benefit decision-making:[8] The focus of this discussion paper is the quantification of the additional uncertainty in hazard and risk assessment that is introduced by replacing testing information by non-*in vivo* testing information. The consideration of uncertainty in predictions from QSARs, which are a form of non-*in vivo* testing information, could improve the way QSARs support chemical safety assessment under REACH. It is argued that it is useful to consider uncertainty in QSAR predictions, as this: a) supports rational decision-making; b) facilitates cautious risk management; c) informs uncertainty analysis in probabilistic risk assessment; d) could aid the evaluation of QSAR predictions in weight-of-evidence approaches; and e) provides a probabilistic model to verify *in vivo* experimental data used in risk assessments. The discussion is illustrated by case studies of QSAR integrated hazard and risk assessment. This study is accompanied by a suggestion for a conceptual framework of uncertainty treatment in QSAR predictions.[9] It suggests that a distinction is made between quantitative and qualitative uncertainty in QSAR predictions, referring to the magnitude of the error and the confidence in the prediction, respectively. It provides guidelines on how the integration of QSARs into risk assessment may be facilitated by including the assessment of predictive error (quantitative uncertainty) and predictive reliability (qualitative uncertainty) into the predictions of the model. An example of an extended uncertainty analysis, in which quantitative uncertainty is enlarged according to the characteristic of qualitative uncertainty, is provided in one of the case studies of Sahlin *et al*.[8]

— The estimation of Species Sensitivity Distributions (SSDs) from uncertain (QSAR-based) effects data:[10] The question addressed in this paper is whether QSAR-predicted toxicities can be included in SSD-based PNEC estimates, and whether any modifications need to be made to account for the uncertainty in the QSAR-model estimates. This problem is addressed from a probabilistic modelling point of view. It is shown that the fitting of a Bayesian model to toxicity data with error-in-data, results in an SSD with a predictive distribution that is an average of posterior spaghetti plot densities, or cumulative distributions. Subsequently, new predictive extrapolation constants are derived for situations where all species data are uncertain with equal errors. It is shown that the consideration of uncertainty in species data results in several improvements over previous median uncertainty log HC5 estimates (i.e. the hazardous concentration at which 5% of the biological species potentially present in an ecosystem are not protected). These are easily calculable from spreadsheet Student-t functions; they are based on a more-realistic uniform prior for the SSD standard deviation; and they are single-number extrapolation constants, which are more sensitive to (low) sample size.

— The implementation of prioritisation and risk assessment of groups of chemicals belonging to a specific chemical class:[11] In this contribution, the example shown is of prioritisation of chemical compounds, polybrominated diphenyl ethers (PBDEs), as implemented in an online web tool that also permits the calculation of the environmental risk of chemical compounds from a web interface. The environmental fate in the aquatic environment is assessed by using the SimpleBox model, while adverse effects on the aquatic environment are assessed by the 2-dimensional Monte Carlo simulation of a QSAR-integrated SSD approach.[8] The case study of QSAR integrated risk assessment of 209 PBDEs demonstrates the treatment and influence of uncertainty in predicted physicochemical and toxicity parameters in probabilistic risk assessment.

Overall, these contributions illustrate all of the essential aspects of ITS, starting with experimental data, focusing on alternatives to *in vivo* testing, addressing the uncertainty of QSAR predictions, demonstrating the integration of non-testing information in probabilistic hazard and risk assessment, and exemplifying the implementation as an easily accessible web tool. The final conclusion of the paper by Tetko *et al*.[11] is "Thus, the developed approach can be easily used for the estimation of risk of new classes of molecules beyond those analysed in the CADASTER project". This premise is exactly what we had in mind when initially designing the CADASTER project.

Acknowledgement

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