CADASTER

CAse studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment

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General

CADASTER is a project that was granted within the 7th Research Framework Programme of DG Research of the European Commission. CADASTER aims at providing the practical guidance to integrated risk assessment within REACH by carrying out a full hazard and risk assessment for chemicals belonging to four compound classes. The main goal is to exemplify the integration of information, models and strategies for carrying out safety, hazard and risk assessments for a selected number of compounds within four specific chemical domains. Real hazard estimates will be delivered according to the basic philosophy of REACH of minimizing animal testing, costs, and time. CADASTER will show how to increase the use of non-testing information for regulatory decision whilst meeting the main challenge of quantifying and reducing uncertainty.

Synthesis of research findings and recommendations for prioritization

Work package 4. Integration of QSARs with risk assessment

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Task 4.5 Policy and management - Deliverable 4.6: Synthesis of research findings and recommendations for prioritization

Summary

The aim of this task was to provide an overview of the implementation of the data, models and tools generated within the CADASTER project in probabilistic risk assessment. More specifically it was aimed to exemplify the quantitative implementation of alternatives to experimental (animal) testing in probabilistic risk assessment on the basis of case studies performed within the project. The implementation includes virtual screening (ranking) and subsequent prioritization of chemicals based on data and models collected or developed within the project together with a multivariate characterization of the applicability domain. Thereupon, prioritization/ranking is performed on the basis of quantitative probabilistic risk assessment taking uncertainties and variability in input data and in model formulations explicitly into account. The actual webtool that is developed to implement the probabilistic risk assessment as develop within the CADASTER project, is exemplified by means of a specific case study and both the technical details of the webtool as well as the corresponding case study are reported. The description of the case studies performed boils down into a number of recommendations and a general implementation strategy regarding the implementation of alternatives to testing in current legislation with a focus on REACH.

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1 Introduction

1.1 General

Authorization and restriction of chemicals (REACH) requires demonstration of the safe manufacture of chemicals and their safe use throughout the supply chain. 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 at closing huge gaps of knowledge on physico-chemical properties and adverse effects of large numbers of chemicals. Thereupon REACH aims to reduce animal testing by optimized 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 in the 1-10 tonne 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 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 – Minimization of the actual numbers of animals used in the required tests, and 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 of above 100 tonnes to minimize animal testing.

Operational procedures are to be developed, tested, and disseminated that guide a transparent and scientifically sound evaluation of chemical substances in a risk-driven, context-specific and substance-tailored manner. The procedures include alternative methods such as chemical and biological read-across, in vitro results, in vivo information on analogues, qualitative and quantitative structure-activity relationships (SARs and QSARs, respectively), thresholds of toxicological concern, and exposure-based waiving. Concerted action and intensive efforts are needed to operationalize all possible alternatives into a workable, consensually acceptable, and scientifically sound strategy for 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. Reasons 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.

CADASTER aims at providing practical guidance to integrated hazard and risk assessment procedures by exemplifying a hazard and risk assessment 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 www.cadaster.eu as an on-line and standalone tool for development, publishing and use of QSAR models for REACH, compatible with the 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 for large numbers of substances by integrating multiple methods and approaches with the aim to minimize 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 that were selected as the chemical classes of choice for CADASTER:

1 – Polybrominated biphenylethers (PBDE), typically being a class of hydrophobic chemicals that pose a threat to man and the environment.

2 - Perfluoroalkylated substances and their transformation products, like perfluoroalkylated sulfonamides, alkanoic acids, sulfonates. Fluorinated compounds are typically a class of persistent, relatively hydrophilic compounds that may be toxic for man and environment.

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

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

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

1.2 Aim of this report

The goals and objectives of CADASTER have been operationalized in the milestones and deliverables as summarized in the Description of Work of the CADASTER project, and all public deliverables are available via the CADASTER website. One of the goals of CADASTER is to provide recommendations on a viable management strategy for optimized testing and in-silico modelling of hazardous organic substances. To operationalize this goal, the aim of this report is to fuse the research findings with other ongoing research and regulatory developments. Synthesis of research findings is often made qualitatively, but here the analysis and fusion of results are driven one step further to a quantitative assessment in which data, models and tools developed and generated within all Workpackages are exemplified.

Apart from exemplifying the application of alternatives to testing in risk assessment with a focus on assessing uncertainty and variability in probabilistic risk assessment, examples will be given in this report of:

- Assessing uncertainty and propagation of uncertainty in environmental fate modelling on the basis of Quantitative Structure Property Relationships (QSPRs) and Quantitative Structure Activity Relationships (QSARs), including QSPR/QSAR-induced uncertainty in overall persistence (Pov) and long-range transport potential (LRTP);
- ranking of compounds from within the four 'CADASTER classes of compounds' on the basis of their environmental hazard;
- ranking of compounds from within the four 'CADASTER classes of compounds' on the basis of their environmental risk, as calculated by combining effect assessment with fate assessment, whilst taking uncertainty and variability in input data into account;

- assessing uncertainties in risk assessment based on QSARs;
- applying read across approaches to chemicals from within a class of compounds of a heterogeneous nature without much structural resemblance across the chemicals that constitute this class (in this example: fragrances);
- prioritization of brominated diphenylethers based on PBT evaluation and uncertainty analysis;
- prioritization of (benzo)triazoles and polyfluorinated compounds based on hazard assessment;
- prioritization of (benzo)triazoles based on risk assessment;

It should explicitly be noted that although all cases studies were performed within Workpackage 4 of CADASTER, they build upon the data and models collected, generated, and validated within Workpackages 2 and 3, which have been made publicly available in Work Package 5 through the QSPR Thesaurus web site http://www.qspr-thesaurus.eu.

Chapter 2 of this report contains an introduction on the integration of alternatives to experimental testing in risk assessment, the case studies that are the core topic of this report are provided in chapter 3. The implementation of probabilistic risk assessment within CADASTER in the QSPR Thesaurus website http://www.qspr-thesaurus.eu is exemplified explicitly in chapter 4 of this report. General recommendations on the main findings of the case studies and the elements of a viable management strategy for dealing with alternatives to experimental testing are provided in chapter 5. Details of the case studies can be found in the appendices to the report.

2 Integration of alternatives to experimental testing in risk assessment

General

When a chemical is manufactured or imported in quantities of more than 10 tonnes per year, it is required to conduct a chemical safety assessment (CSA) and to prepare a chemical safety report (CSR). This chemical safety assessment generally is achieved along two separate lines of evidence: Hazard Assessment (HA) and Exposure Assessment (EA). Exposure assessment is to be carried out when a substance is classified as dangerous, or when a chemical is assessed to have PBT or vPvB properties (PBT - Persistent, Bioaccumulative, Toxic; vPvB - very Persistent and very Toxic). The two assessments are integrated in the Risk Characterisation (RC) stage of the chemical assessment, as shown in Figure 1 (ECHA, 2008a). In the Risk Characterisation phase, a central role is played by the Risk Characterisation Ratio (RCR).

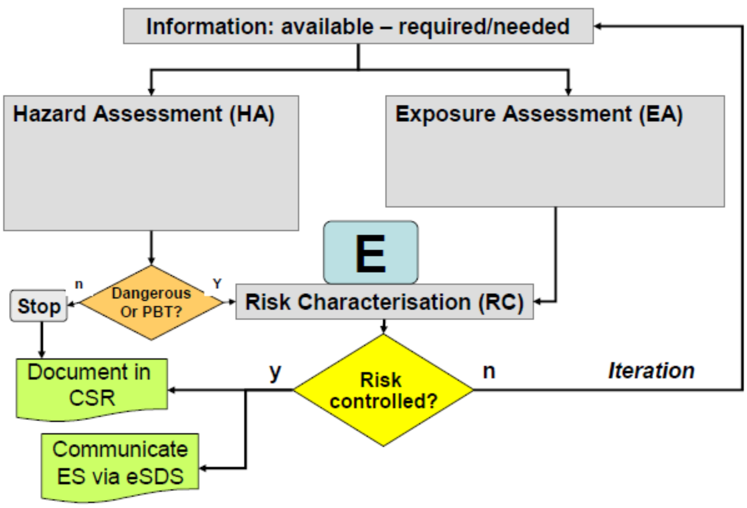

Figure 1. Information flowchart of a Chemical Safety Assessment. Lines of evidence through Hazard Assessment (HA) and Exposure Assessment (EA) meet at the Risk Characterisation (RC) stage that may either end the assessment, or lead to further iterations of the assessment (ECHA, 2008a).

Use of the RCR implies that chemical risk assessment within REACH basically boils down to comparing (predicted) environmental concentrations (PECs) with (predicted) no-effect concentrations (PNECs), with risk of adverse effects being proportional to the extend in which PEC exceeds PNEC (ECHA, 2008a). The RCR is the ratio of an exposure value and a no-effect value (ECHA, 2008a):

$$RCR = \frac{PEC}{PNEC}, \text{ or } \frac{Exposure}{DNEL}, \tag{1}$$

When sufficient experimental toxicity data are available that cover a range of biota of various trophic levels and a range of (sensitive) endpoints, PNEC values are usually calculated by means of species sensitivity distributions (SSD; Posthuma et al., 2002), in which the available toxicity data are commonly plotted as a cumulative frequency distribution. An example of a SSD is given in Figure 2.

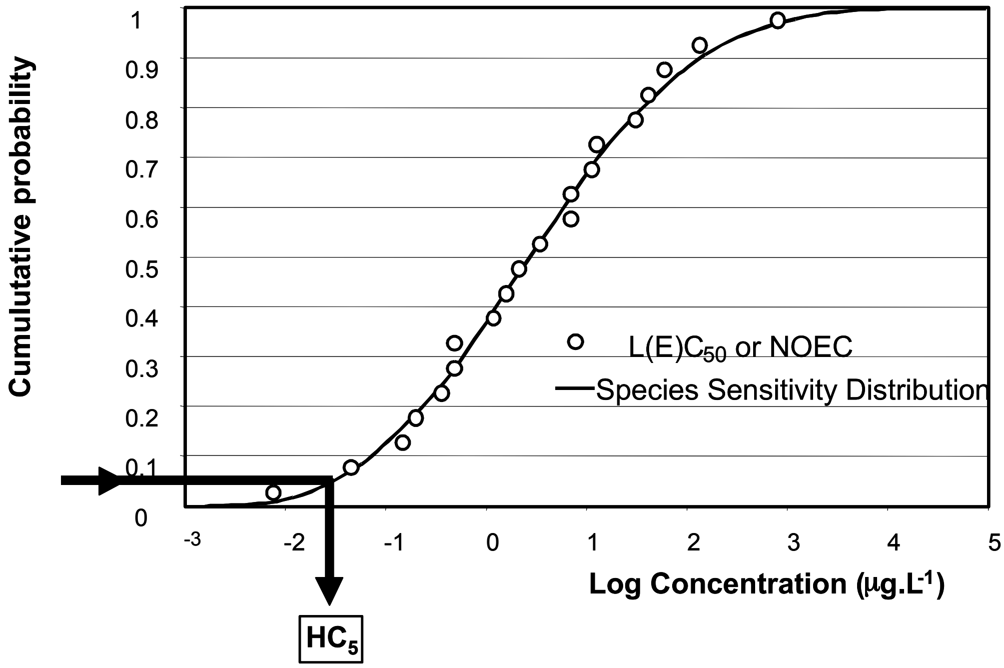

Figure 2. A typical example of a species sensitivity distribution (SSD). Dots represent individual toxicity data, HC_5 = Hazardous concentration at which 5 % of the species is potentially affected.

Species sensitivity distributions are preferably based on experimental data, but in common practice often insufficient experimental data are available to establish the minimum set of data needed to derive a SSD. Variance alternatives are available to supplement existing data or even to substitute for lacking toxicity data, 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 (Van Leeuwen et al., 1996; Bradbury et al., 2004), the concept of Intelligent (or: Integrated) Testing Strategies (ITS) was developed to optimize the integration of available experimental data and alternative means of assessing adverse effects, whilst adhering to one of the main objectives of REACH of minimizing the use of test animals. 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. Intelligent testing strategies are integrated approaches comprising of multiple elements aimed at speeding up the risk assessment process while reducing costs and animal tests (Bradbury et al., 2004). Within an ITS, all alternatives to experimental testing are integrated, as schematically exemplified in Figure 3.

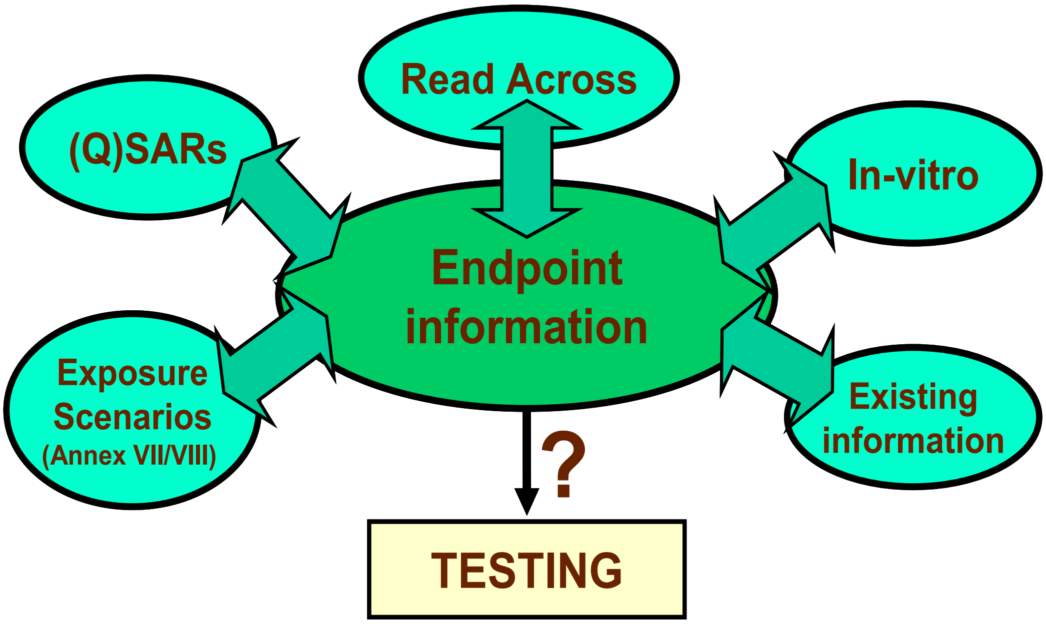

Figure 3. Elements of an Integrated Testing Strategy.

As indicated in Figure 3, 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 (QSAR or QSPR);
- 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 at concentrations well below the no observed effect level.

Implementation of ITS in hazard assessment boils down to what is sometimes called the 3 R's strategy of replacement, reduction and refinement of toxicity testing. Initially though a 7-R strategy was advocated, including the initial 3 R's:

1. Risks based strategy: Focus on risks (include exposure);

2. Repetitive: A tiered approach should be applied, going from simple, to refined or comprehensive (if necessary) to quickly assess chemicals of low concern and to prevent animal testing;
3. Relatives: The focus should be on families or categories of chemicals (a group-wise approach) using read-across, QSARs and exposure categories: move away from the chemical-by-chemical approach;
4. Restriction of testing (waiving of testing) where possible and carry out in-vivo testing where needed in order to prevent damage to human health and/or the environment;
5. Replacement (substitution);
6. Refinement: Reduce suffering and distress of test animals;
7. Reduction of animal testing.

It should be noted on forehand that within the CADASTER project, the focus has been on the implementation of group-wise approaches to hazard and effect assessment of specific, pre-defined, classes of chemicals (i.e. focus on Bullet 3 in the list given above: Relatives). Thus, the focus was on PNEC assessment. In order to meet the CADASTER objective of risk assessment or risk characterisation of chemicals of the specific classes that are the topic of investigation within CADASTER, a standardized emission scenario was applied in view of the lack of detailed information on environmental emissions to derive PEC.

Non-testing approaches in Environmental Risk Assessment

REACH as well as other chemical legislations advocate the use of alternative non-testing approaches. Up till now, however, the acceptance of results derived by these approaches by relevant stakeholders is only limited. In part this is due to insufficient examples being available of successful implementation of alternatives to experimental testing, and clearly more substantial information/evidence needs to be made available to exemplify the application of alternative methods. A change in mindset among all the relevant stakeholders is needed as risk assessment should move from a labour-intensive and animal-consuming approach to intelligent and pragmatic processes.

In silico methods such as (Q)SAR approaches seek to predict the intrinsic properties of chemicals by using various databases and theoretical models. Based on knowledge of chemical structure, QSAR quantitatively relates characteristics of the chemical to a measure of an activity (e.g. human toxicity or ecotoxicity). QSAR models as a tool to reduce experiments with fish or birds for acute toxicity endpoints or bioaccumulation have been developed and validated by European research labs (often within EU-research framework programmes). Examples include the freely available OECD QSAR

Toolbox (http://www.qsartoolbox.org), the ChemProp Database (http://www.ufz.de/index.php?en=6738), the On-line CHEMical Modelling environment (http://ochem.eu) as well as the QSPR Thesaurus web site (http://www.qspr-thesaurus.eu) developed within the CADASTER project. Procedures to validate QSAR models have been developed which are applicable both in the field of human and environmental risk assessment.

Read-Across and grouping are methods to fill data gaps in which information on specific endpoints from one or more chemicals is used to predict the same endpoint for another chemical which is considered to be structurally similar, and hence is expected to have similar physico-chemical properties or similar adverse effects. Applying the group concept means that the physico-chemical properties, mode of action, human health effects or environmental effects or environmental fate may be predicted from data available for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). These similarities may be based on common functional groups, common constituents of chemical classes or others and the assignment of missing properties is performed on the basis of the knowledge of other members of the family.

Weight of evidence (WoE) is an evidence-based approach, involving an assessment of the relative weight of different pieces of information available from previous investigations. To this end, a value needs to be assigned to each piece of information either in an objective way using a formalized procedure or by using expert judgment. The weight given is affected by various factors such as the quality of the data, consistency of results, nature or severity of effects, relevance with respect to the regulatory endpoint, etc. To build a weight of evidence case, information is gathered from all possible sources including literature, read across, (Q)SAR predictions, data from existing in vivo and in vitro studies, (eco)epidemiological data, etc.

Data waiving or adaptation is also accepted by REACH legislation to avoid unnecessary experiments, including animal testing. Adaptation means the use of non-standard methods (e.g. QSAR, grouping and read across) for filling the information gaps. Waiving of the information requirements for an endpoint means that the submission of the standard information for the particular endpoint is not considered necessary in a specific case (e.g. when testing is technically not possible, a well-known example being aquatic toxicity testing of highly hydrophobic organic chemicals which often have a water solubility that is well below their adverse effect levels).

Despite the recognized importance of testing alternatives, also limitations of non-testing approaches in Environmental Risk Assessment (ERA) need to be recognized. The most common limitations include:

- A lack of existing toxicity data for species and endpoints relevant for ERA for in silico model building;
- Low or difficult to ascertain quality of available toxicity data;
- Models for toxicity mechanisms other than acute narcosis and specific endpoints such as chronic toxicity or receptor-mediated effects (e.g. endocrine disruption), are much less developed. This hinders extrapolation of test results (read across) as well as in silico model building;
- For some specific mechanisms (e.g. reactive toxicity), descriptors of chemical structure have been characterised, but their application in practice is still not common;
- Applicability domains of in silico models are often not clearly defined.

These limitations may be translated in a number of research needs:

- Different database entries for toxicity (e.g. NOEC vs. EC_x) are not consistently robust, and their use for QSAR often leads to different models and results. Further characterization and validation of existing toxicity data is urgently needed;
- (Eco)toxicological data submitted to ECHA or other regulatory bodies should be explored and made available for the purpose of in silico model development;
- Determination of the mechanisms of action and its propagation to apical endpoints would be a major breakthrough for advancement of non-testing approaches;
- Understanding the mechanisms can be considered as a pre-requisite for appropriate grouping and QSAR development. Research is needed to identify the most relevant mechanisms and to derive reliable effect data for the development of mechanistic knowledge-based models.

On top of these research needs, there is a need for validation of models and hazard/risk assessment approaches:

- Validation schemes already exist, but they still need to be improved – especially for standardized reporting, where clear guidance with regard to the OECD guidelines is needed;
- Validation may also be a problem for some individual QSAR models, which are incorporated in complex existing tools such as ECOSAR;

- Appropriate training and understanding of personnel is required, particularly of those dealing with registration dossiers. It is of prime importance to inform and educate regulatory authorities so they understand possibilities and limitations of non-testing approaches.

Probabilistic risk assessment and uncertainty analysis

When dealing with non-testing approaches in ERA it should be realised that experimental (standard) data have the highest priority when drawing conclusions on regulatory endpoints. Non-standard information is particularly useful where it can help to avoid an assessment on the basis of invalid or missing experimental data (Ahlers et al., 2008). Testing information is strong in the sense that we are confident in the use of testing information to support decision making. The use of alternatives to testing means that experimental data is replaced by a predictive model. Such non-testing information is less strong than testing information and depends on our confidence in the predictive model. This means that using alternative methods in risk assessment not only introduces uncertainty related to a prediction, but also alters the strength of the background information. It is currently not clear how to treat both these aspects in the probabilistic risk assessment that is common practise in general nowadays as well as in the CADASTER project. However, it is for sure that probabilistic risk assessment requires a quantification of uncertainty. This is to be understood and interpreted in relation to the available background knowledge, which implies that the characterisation of uncertainty rests upon assumptions and decisions taken by the risk assessor.

There are solved and unsolved issues on how to treat uncertainty when going from testing to non-testing information in probabilistic risk assessment. Risk assessment is a tool to describe uncertainty in unknown quantities (Aven, 2010b). Uncertainty in exposure and effects of chemicals is therefore to be given a proper and transparent treatment (Verdonck et al., 2005; National Research Council, 2009). Environmental risk assessment usually distinguishes variability, i.e. natural variation that cannot be reduced by adding more information, from uncertainty. Epistemic uncertainty (i.e. knowledge based uncertainty) is different from variability (stochastic uncertainty or population level), the latter an inherent property of a quantity or system that cannot be reduced by making more observations or gaining more knowledge. Probabilistic risk assessment quantifies uncertainty by probabilities. To complicate things, there are different kinds of probabilities. For example, probability can be seen as expressing a subjective belief or relative frequency that something occurs. Under severe epistemic uncertainty, probabilities have been criticized for being too precise, which has led to the use of alternative non-probabilistic treatments of uncertainty in risk assessment. If a risk assessor's uncertainty due to lack of knowledge and systematic measurement errors (partial ignorance and epistemic uncertainty) is adequately quantified by probability, a major advantage is that its results in an interpretable decision support (Aven, 2010a). There is currently an ongoing discussion on the meaning of probability and the use of probabilistic, non-probabilistic or hybrid approaches, which may be of less interest, but nevertheless useful to be aware of, to researchers in empirical sciences. The use of alternative methods in a probabilistic risk assessment framework thus leads to questions about the reliability of non-testing versus testing information, the treatment of uncertainty associated to predictive models used to inform input parameters to computer models, and the impact that non-testing information and its uncertainty may have on decision making.

Treatments of uncertainty can be qualitative (tier 1), deterministic (tier 2) using point estimates and worst case assumptions, and probabilistic (tier 3). Probabilistic risk assessment includes analysis of the uncertainty in a risk assessment, followed up by an analysis of the sensitivity of risk to different sources of uncertainty. Uncertainty in probabilistic chemical safety assessment can roughly be divided into three categories: parameter uncertainty, model uncertainty and scenario uncertainty. Scenario uncertainty is "the uncertainty in specifying the scenario(s) which is consistent with the identified use(s) of the substance" and is of less relevance for the uncertainty related to an alternative method.

The ECHA guidelines (ECHA, 2008b) make the following description of model and parameter uncertainty:

> **"Model uncertainty** is the uncertainty in the adequacy of the model used with the scope and purpose of the assessment. In risk assessment, mathematical and statistical models are often applied to represent an exposure or hazard process though a model is always a simplification of reality. Model uncertainty is principally based upon extrapolation (i.e. use of a model outside the domain for which it was developed), modelling errors (i.e. non-consideration of parameters in the model structure itself, assumption of well-mixed phases etc.) and dependency errors (i.e. lack of consideration of correlations between parameters).
>
> **Parameter uncertainty** is the uncertainty involved in the specification of numerical values. Risk assessment involves the specification of values for parameters, either for direct determination of the exposure/effect or as input for mechanistic, empirical or distribution based models which are used. The uncertainties surrounding these values are very common due to lack or insufficiency of data.
>
> Parameter uncertainties include:

- Measurement errors: e.g. influence of the methodology used, errors in the analytical method used to measure chemical concentration, technical inadvertence;
- Sample uncertainty: representativeness of the data set, e.g. a small sample may not give the entire range of values found in reality; the sample may be biased towards lower or higher values as a result of the selection criteria used to take the sample; averaging methodologies;
- Selection of the data used for assessing the risk: i.e. use of default data (e.g. TGD default data are frequently used for exposure assessment) or choice of the dose descriptor (i.e. uncertainty in choosing one data among others for risk assessment purpose);
- Extrapolation uncertainty: i.e. use of alternative methods (e.g. QSAR, in-vitro test, read-across for similar substances) or use of assessment factors (e.g. inter-species, intra-species, acute to chronic, route to route, lab to field extrapolation).

The separation of parameter and model uncertainty to characterize uncertainty in QSAR predictions is not frequently used in modelling. Instead a distinction lies between predictive uncertainty and predictive reliability (Sahlin et al., 2011). According to Sahlin et al. QSARs applied in risk assessment or decision making result, apart from the uncertainty associated to the magnitude of the error in a QSAR prediction, in two kinds of model uncertainties in need of treatment; the reliability in using a model for prediction (also known as confidence in prediction) and the consideration of alternative QSAR models to predict the same endpoint (e.g. consensus modelling by model averaging). The treatment of model uncertainty in a QSAR contributes the treatment of uncertainty in the parameter that the QSAR is supposed to predict. Thus from the perspective of an assessment model, is uncertainty in a QSAR prediction a parameter uncertainty. Uncertainty in predictions is part of the prediction and is a major concern, especially when predictions may influence the safety of environmental systems. In conflicting cases it is important that the assessments of uncertainty in model predictions like QSARs can be motivated and reflect the assessors uncertainty in a prediction. For a more detailed guidance on treatment of parameter uncertainty in uncertainty analysis and of the characterization and propagation of predictive error and predictive reliability (see below) related to QSARs when applied in probabilistic risk assessment we refer to the CADASTER deliverable D4.2.

Predictive uncertainty

Applying QSARs in risk assessment raises the need to consider uncertainty in predictions and the accuracy of a QSAR prediction in relation to the intended use of the QSAR (ECETOC, 1998). While QSARs are based on data that are variable (e.g. due to measurement errors or variability), the product of the QSAR is reported as a point estimate (Walker, 2003). In a recent overview of current practice to characterize uncertainty in QSAR predictions Sahlin et al (2011) found that current QSAR practice include several approaches to assess parameter uncertainty (roughly divided into expert judgment, estimates based on re-sampling and assessments based on probabilistic modelling), but that the integration of QSARs in risk assessment would benefit from probabilistic QSARs in which uncertainty is quantified by probabilities. The need of probabilistic models was pointed out by Walker et al. (2003a) who suggested

> *"that errors needs to be evaluated when applying QSARs by providing confidence intervals that take into consideration the uncertainty associated with the estimate"*.

This phrase implies first of all that if a confidence interval can be calculated there must be an underlying probability distribution (parametric or non-parametric) and this is what shall be used to the describe the parameter uncertainty when the QSAR provides an input parameter to a probabilistic risk assessment. Further, if it is implicit that the confidence interval should cover the actual value with a certain degree of confidence, it presumes a Bayesian interpretation of uncertainty. There is no problem having a Bayesian approach to uncertainty, this is in fact the way that risk assessment usually deals with uncertainty (Aven 2010a). The Bayesian approach is to regard a model as uncertain and let the combination of prior belief and the QSAR training data lead to a posterior belief in what QSAR models that most likely describe observed data. Taking the average over the posterior means gives a point prediction, while the full posterior distribution provides an estimate of the uncertainty in prediction (Obrezanova and Segall, 2010). A predictive distribution is the posterior of observables as opposed to model parameters.

A prediction can be a result of several QSAR models. QSARs can be built on different algorithms for supervised learning and divisions into training and validation data sets. It has been shown that consensus averaging frequently provides better results compared to the use of individual models (Tetko et al, 2008; Zhu et al, 2008, Sushko et al, 2010). Model Averaging is a technique for consensus modelling of an ensemble model developed on the same training data set, or validated on the same external test set. A test set is a set of chemicals, not present in the training set, that is used to validate (assess the predictive ability of) a QSAR. Model Averaging is a weighted average of predictions where each weight is assigned by some measure of performance based on the common data set using measures of divergence such as Kullback-Leiber divergence, Akaike weights or Bayes factor (Johnson and Omland, 2004). Model averaging is a way to deal with model uncertainty in the probabilistic risk assessment via the characterisation of the predictive distribution. Predictions from several QSARs (e.g. local and global models) can alternatively be combined based on expert judgment.

Here it is appropriate to add some comments on predictive error, which is measure describing the distance (error) between a point prediction and the actual value. Predictive error is not a fixed value. It changes from compound to compound. For example, predictive error ought to increase with the extent of extrapolation (Tetko et al, 2008). This holds even for models where errors are assumed to be equally distributed. The extent of extrapolation is one factor that influences a models predictive reliability, i.e. the reliability in using this particular QSAR to predict a particular chemical compound.

Predictive reliability of models

The acceptability of QSAR predictions depends on the regulatory endpoint regarded (Ahlers et al., 2008; ECETOC, 2003). Greater confidence is based on models of acute effects compared to chronic ones and on models of baseline toxicity compared to predictions based on specific modes of action or chemical classes showing more than baseline toxicity. Determining which QSAR models are suitable for regulatory purposes is not the focus in this report, and we refer to existing literature (Gramatica, 2007). Predictive reliability, or confidence in prediction, is a statement of the strength of non-testing information as part of the background knowledge. In relation to an experimental test, is a QSAR prediction information of a lower strength, since it is not a direct empirical observation of the activity or property. It is relevant to ask in what way the lower strength of non-testing information can be considered in the probabilistic risk assessment. Overconfidence in a QSAR to produce reliable predictions can be avoided if the assessor is aware of, if, how and why the QSAR was developed and validated. There is a need to understand the limitations of chemical structure representation, descriptors, statistics, data sets, endpoints, and variability of measured data. In order to maintain reliability it has been suggested to test the acceptability of QSARs by the so called OECD principles (OECD, 2006).

The chemical domain for which a QSAR has been built is an important factor to evaluate the reliability of a model, by looking to what respect a compound to be predicted falls inside the applicability domain (Clark and Waldman, 2013). The applicability domain is a region in chemical space determined by the training set and (but less clear) by the model. There is a danger in treating a QSAR model as a black-box. De Roode et al. (2006) showed that QSARs are not always in the models domain of applicability and the accuracy of prediction is low in such cases. Given that the OECD principle of a defined domain of applicability is fulfilled, predictive reliability must be evaluated in every situation where a QSAR model is applied for prediction (Gramatica, 2007; Gramatica et al. 2012). Predictive reliability of a QSAR should be judged both globally (average) and locally (item-specific). Global measures such as confidence index based on crucial factors influencing the confidence of a computation model of toxicity used to compare models (Schultz et al., 2004), do not say anything about how the confidence in predictions varies between items to be predicted, i.e. does not provide local and item-specific reliability. There are attempts to assess predictive reliability by sensitivity analysis and a shown correlation between a measure of the applicability domain and the assessed predictive reliability (Bosnic and Kononenko, 2009). Even though such correlations have been found, it is without any further elaboration difficult to integrate such qualitative statements in a probabilistic risk assessment.

Important questions are whether a compound lies inside the models domain of applicability, and if the associated uncertainty to a prediction following from predictive inference reflects our confidence in the prediction. Aspects of predictive reliability can be dealt with by flagging (i.e. put it down in the risk report but use the QSAR prediction as it is), go for other non-testing information (maybe in combination with the QSAR prediction), or let it be reflected in the parameter uncertainty followed by sensitivity analysis. Uncertainty due to extrapolating outside the applicability domain can be dealt with by enlarging parameter uncertainty by some uncertainty factor. Ahlers et al (2008) suggest that when the amount of information gathered remains somewhat below the standard requirements, it might be preferable to increase the uncertainty factor instead of performing a missing test. If the higher safety factor results in no apparent risk, further testing may be avoided and animals may be saved. For example (from Ahlers et al 2008) if EC50 values for daphnia and algae and a QSAR estimate for fish are available and the PEC/PNEC ratio is very low, a fish test may not be necessary (waiving of testing); whereas a chronic fish test should be considered directly when the PEC/PNEC ratio is high. Thus, sensitivity analysis is a helpful tool to evaluate whether a QSAR prediction can be used or not. The influence of QSAR prediction is not only related to the accuracy of the prediction itself, but depends on how the uncertainty in the prediction propagates in the assessment model, which depends on number of times the parameter is used and if it reduces or increases the assessed risk (Walker, 2003).

Predictive reliability can be assessed in several ways, roughly divided into measures of extrapolation and measures of performance. The former includes various metrics of the applicability domain (Netzeva et al., 2005). Performance measures includes non-probabilistic performance measures, such as standard deviation in ensemble predictions, uncertainty measures, such as locally assessed predictive errors, and probabilistic performance measures, such as local coverage (hit rates or empirical confidence levels). For example, the variation between ensembles of predictions is a measure of predictive reliability but not an estimate of predictive error per se. However, it can be correlated to predictive error, since items for which predictions differ between models most likely are given less reliable predictions.

Assessments of predictive uncertainty and predictive reliability have been carried out in WP4 in the CADASTER project. There are approaches that use non-parametric bootstrap based upon an assumption of a positive correlation between reliability and predictive error. A nice feature with bootstrapping is that the assessment of reliability dependent predictive errors does not need external data. Instead, predictive errors are calibrated using n-fold cross-validation, e.g. bagging approaches (Tetko et al., 2008; Sushko, 2010; Sushko et al., 2010a) or locally assessed Predictive

Error Sum of Squares (manuscript in review). The assessment of predictive errors is done according to the concept of "distance to model", which is a generalized idea of a similarity of a tested molecule to the training set molecules. The concept has been further extended for classification models (Sushko et al., 2010a; Sushko et al., 2010b). A complete description of the analysis and discussion of the concept "distance to model" is found in the PhD thesis of I. Sushko (2010).

A decision maker may be interested in the consequences the model usage may have on the accuracy of the risk assessment. To this end, a useful measure of predictive reliability is the probability of a prediction being wrong (i.e. 1 – probability of being accurate). The probability of having an erroneous prediction for compounds at the border of the AD can assist in the consequences of being on the border. In this respect, many of the measures of reliability fail as they are not probabilistic. Conan statistics provide different measures related to the probability of making different kinds of errors and are applicable when the prediction is a classification. For classification models specificity and sensitivity and the uncertainty in the probability of being in one class or the other may vary over the applicability domain. For regression models, even though predictive reliability ought to decrease, and predictive error ought to increase, in regions of the applicability domain where the model is less defined, it does not necessarily imply that a prediction having a larger predictive error must be less reliable. We therefore avoid using the predictive error (as it is) to characterize the accuracy in using a model for prediction (predictive reliability). Of importance is if the prediction and its associated uncertainty cover the actual value (Sahlin, 2012). There is a relationship between the domain of applicability and predictive error (Weaver and Gleeson, 2008), but the change in predictive error may not be large enough to reflect the reduced reliability in model predictions. Instead we argue for the use of confidence (empirical confidence, but see also tolerance intervals) that reveal how well we believe the predictive distribution is expected to cover the actual value. Tong et al. (2004) assessed coverage (i.e. accuracy estimated as the number of compounds that fell inside the corresponding prediction interval) for a given confidence level over different regions of the AD defined by extrapolation measured by the proportion of items in the training data set that are further away than the item to be predicted. Coverage was lowest for the most extreme region of the applicability domain. It is however difficult to get good measures of predictive performance in the most extreme regions as there is by definition few data points there.

The probability of committing different types of errors to guide decision making whether the risk assessment is reliable or if it is worthwhile to reduce the probability of being wrong in some of the input parameters. This is easiest to understand for a classification models (a test) and where the outcome of the test directly influences the decision (Jaworska et al., 2010). The decision to test or not is directly seen whether a test have an increase in the expected utility (or decrease in expected loss). However, it is difficult to derive how the probability of an input parameter of being wrong propagates through a risk assessment models, such as SIMPLEBOX. The option is instead to study the influence that uncertainty in an input parameter has on the overall uncertainty of the assessed output. For example, will a reduction of the uncertainty change the decision by moving a critical value such as the 95th percentile of the PEC/PNEC ratio over a decision threshold?

When the extent of extrapolation is judged as unacceptably high the recommendation could be:

- Do not use a QSAR if the compound to be predicted is an unacceptable extrapolation.

Alternatively one could use the QSAR but

- Flag that the compound is extrapolated and in a sense judged as being outside the statistical applicability domain by reporting the extent of extrapolation from the QSAR training data set.

- Add extrapolation uncertainty to the predictive uncertainty derived by predictive inference.

- Combine QSAR prediction with other non-testing methods.

Read Across is a frequently used non-testing method in the OECD toolbox. Both QSAR and Read Across predict by analogy, where the major difference is that QSARs are based on a learning algorithm and asses the uncertainty or accuracy in predictions based on empirical data, whereas uncertainty or accuracy in read across is if at all derived by expert judgment. Alternatively a Read Across is an extreme kind of local QSAR.

In all these cases is it important to communicate the lowered reliability in the prediction ("AD" statement in Figure 4) in the risk assessment report (example in Figure 5). A recommendation is to follow up the use of a parameter with associated low reliability with a sensitivity analysis of its impact on the regulatory decision.

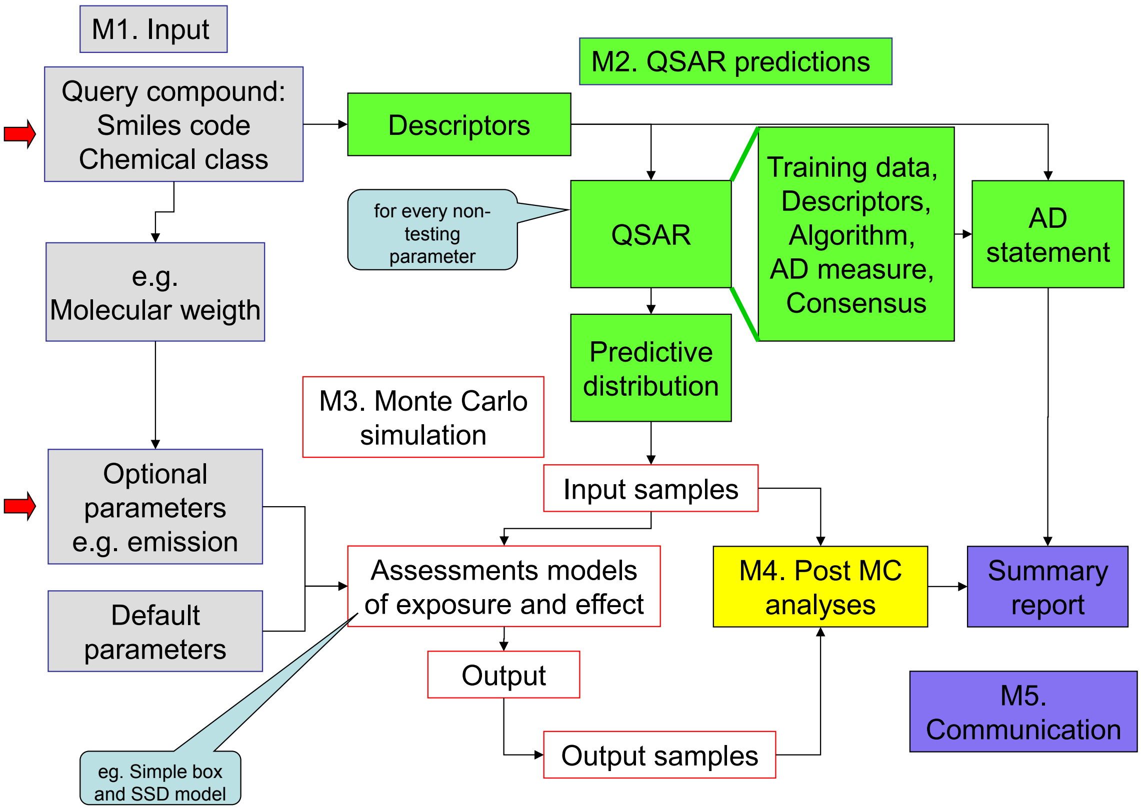

Figure 4. Workflow for QSAR-based risk assessment, AD = Applicability Domain.

3 Integration of alternatives to (animal) testing in regulation – implementation of CADASTER case studies

3.1 Overview of activities

The current regulatory developments within REACH outlined in chapter 2, with a focus on the integration of alternative methods in a probabilistic risk assessment framework, have provided important considerations in the initial design of the CADASTER project. CADASTER is aimed at exemplifying the integration of the various alternatives to experimental testing and the consequences for environmental risk assessment, explicitly considering uncertainties associated with the use of alternatives to replace experimental data. In line with its acronym, the project is designed to provide case studies on the development and application of in-silico techniques for environmental hazard and risk assessment, and to use the project results as an illustration of how to deal with the major limitations and uncertainties related to the implementation of alternatives to testing in hazard and risk assessment. To this end, and as highlighted above in terms of major limitations and research needs, the following activities were amongst others performed:

- Collection of existing data and predictive models on the endpoints that are essential for performing hazard and risk assessment of chemicals within REACH (Aim of this activity: filling the research gap identified above of lack of data for endpoints relevant for ERA and for *in silico* model building);
- Exploring and making available of (eco)toxicological data, amongst others for the purpose of *in silico* model development (Aim of this activity: filling the research gap identified above of making data available for *in silico* model building. It should be noted that more or less by definition it is not possible at this stage to fill in the whole of the research gap identified above of making data available that is submitted to ECHA and other regulatory bodies);
- Assessing the quality of available toxicity data (Aim of this activity: filling the research gap identified above of low or difficult to ascertain quality of available toxicity data, and need of characterization and validation of existing toxicity data);
- Supplementing existing experimental data to allow for *in silico* model development for endpoints essential for risk assessment, and to allow for validation of existing and newly developed models (Aim of this activity: filling the research gap identified above of lack of data and models for endpoints relevant for ERA and for *in silico* model building);

- Collection of existing alternatives to experimental testing, with a focus on QSAR models on the endpoints that are essential for performing hazard and risk assessment of chemicals within REACH (Aim of this activity: filling the research gap identified above of lack of models for endpoints relevant for ERA);
- Development of new (QSAR) models and validation of existing and newly developed (QSAR) models, including development of consensus models (Aim of this activity: filling the research gap identified above of lack of models for endpoints relevant for ERA);
- Implementation of tools to estimate the applicability domain of models and to optimize experimental design. This activity includes characterisation of variability and uncertainty of models and underlying, and sensitivity analysis of individual models (Aim of this activity: filling the research gap identified above of lack of definition of the applicability domain of models);
- Development of a computational framework for QSAR based probabilistic risk assessment, including uncertainty analysis of the risk characterisation ratios (Aim of this activity: filling the research gap identified above of need of probabilistic risk assessment);
- Development and public release of the QSPR-THESAURUS Website and associated databases containing all data and models made collected and generated within the CADASTER project (Aim of this activity: filling the research gap identified above of lack of robustness of different database entries for toxicity, consequently leading to different QSAR models and results);
- To improve and validate individual QSAR models, and prepare standardized reporting formats for the models (like QMRF – the QSAR Model Reported Format developed by JRC and implemented in the OECD QSAR Toolbox). Aim of this activity: filling the research gap identified above of lack of validation of individual QSAR models which are incorporated in complex existing tools such as ECOSAR);
- Perform training to risk assessors, national chemicals authorities (particular from Eastern European countries), industry and SMEs on the use of alternative tools for risks assessment in REACH, amongst others demonstrating how the tools developed within CADASTER as well as the models available in the OECD QSAR toolbox can be used to estimate REACH end-points for chemical compounds and thus decrease the number of animal tests. This activity included training on how to develop new models for the assessment of REACH-end points (in particular for new scaffolds of compounds for which there are no reliable QSAR models) and how to use the software developed by the CADASTER project participants (Aim of this activity: filling the gap identified above of need of appropriate training and understanding of personnel, particularly of those dealing with registration dossiers);

The core of the activities was directed towards the following topics:

- Collection and generation of fate and effect data essential for risk assessment;
- Collection and development of predictive models for endpoints essential for risk assessment;
- Development of methodologies for assessment of the applicability domain of models;
- Making data and models available to any outside user via the project websites www.cadaster.eu and http://www.qspr-thesaurus.eu.
- Characterisation of uncertainty, variability, model sensitivity;
- Training of (future) risk assessors and outreach of project results;

3.2 Case studies

Introduction to case studies

As already stated above, the case studies were especially aimed at exemplifying the use of alternatives to experimental testing and to assess the uncertainties and variability in probabilistic risk assessment related to the use of alternative methods. In all cases, a general overview is given of the study performed and the main conclusions drawn. The overviews are translated in general recommendations regarding the use of alternative methods in risk assessment. An important aspect of the case studies was the characterisation of uncertainty, as performed within workpackage 4. Uncertainty of QSARs as identified in workpackage 2 (activity: collection of models) and further assessed in workpackage 3, have been characterized both qualitatively (e.g. as the confidence in individual predictions) and quantitatively (by a probability distribution for the error compared to experimental obtained estimate). The characterization of uncertainty has been made possible by suggesting a conceptual framework to define and understand uncertainty in a QSAR prediction in a decision context and to provide a framework for the theoretical understanding of approaches to assess uncertainty in predictions covering probability models and principles for statistical inference (manuscript submitted to the proceedings of the second workshop CADASTER in ALTA). Focus has been to derive a conceptual framework for predictions for QSAR regressions (i.e. with a continuous as opposed to a categorical response) which is common within environmental risk assessments and for which uncertainty is less often reported probabilistically. The contribution of individual QSAR predictions to the overall risk assessment framework has been evaluated by sensitivity analyses in a set of QSAR integrated fate and effect assessments, as depicted in the case studies that are included in this report. Modelling of variability was performed with regard to the application of QSARs in hazard assessments, based on the approaches of applying Assessment Factors and on Species

Sensitivity Distributions (SSDs). Current guidance for QSAR integrated probabilistic risk assessment together with actions taken within CADASTER to address identified gaps in current guidance are provided in Deliverable 4.2.

Case study 1 – Uncertainties in triazole risk assessment based on QSA(P)Rs

An important aspect of probabilistic risk assessment is the assessment of the impact of uncertainties in input parameters on the outcome of the risk assessment. In this specific study, the sensitivity of risk assessment to uncertainties associated with the use of QSARs (including QSPRs) was investigated. Triazole fungicides were taken as the compounds as investigation and apart from quantifying uncertainties in risk assessment due to the use of QSARs, also uncertainties related to the use of QSARs in assessing the potential for long range transport and the persistence of triazoles were assessed. The case study is included in Appendix 1.

Summary of the case study

The risk assessment of triazole fungicides is hampered by a lack of monitoring and toxicity data. The goal of this case study was to determine the influence of the use of quantitative structure-activity (property) relationships (QSARs) on the uncertainty in the risk assessment of a selection of triazoles. Soil sorption partition coefficients, solubility, melting point, vapor pressure and hydroxyl radical reaction in air were predicted with QSARs; biodegradation rates were predicted by combined use of semi-quantitative ratings and experimental half-lives; and no effect concentrations were predicted with QSARs. All data were implemented in the multimedia fate model Simplebox. Parameter uncertainty was treated as a probability distribution, and assessed using statistical methods propagated by Monte Carlo Analyses.

Conclusions of the case study

We studied the influence of the use of QSARs on the uncertainty in the outcome of a risk assessment for triazoles, and determined the relative contribution of the different predictive models to the overall uncertainty. The typical maximum permissible emissions to agricultural soil were highest for Bromuconazole and Difenoconazole, i.e. 2.09 $\bullet 10^6$ and 2.26 $\bullet 10^6$ kg/day, respectively, with 90%-Confidence Intervals of four orders of magnitude. For Tebuconazole, Triazamate, and Metconazole we found lower typical Maximum Permissible Emissions, that is between 5.15 $\bullet 10^4$ and 8.00 $\bullet 10^4$ kg/day, with 90% Confidence Intervals ranging three to five orders of magnitude. We found that the uncertainty of the maximum permissible emission to agricultural soil was mainly determined by uncertainty in the QSPR soil sorption partition coefficient, in the QSAR for biodegradation in water, and in the QSAR for toxicity to different species. In this case three predictions were outside the applicability domain of the corresponding QSARs. Nevertheless, the risk assessment performed in this study may still be reliable if it can be shown that uncertainty in these parameters has a negligible influence on the uncertainty of the Maximum Permissible Emission.

Case study 2 – Non-testing versus testing based risk assessment of polybrominated biphenyl ethers (PBDEs)

In this case study, the outcome of the risk assessment of PBDEs is evaluated for cases in which non-testing information is used as compared to the use of non-testing information for the same PBDE-congeners. The comparison explicitly takes typical uncertainties in input parameters into account, with a focus on uncertainties in effect/hazard assessment (PNEC). The case study is included in Appendix 2.

Summary of the case study

Exposure (PEC) and effect (PNEC) were assessed based on QSPR and QSAR predictions for PBDEs. Whenever available, parameters for the exposure assessment and species effect concentrations were replaced by experimentally tested information. Unfortunately, did none of the chosen PBDEs have testing versus non-testing based assessments of both exposure and effect, only one at a time. Using QSPR-based exposure assessments introduced more uncertainty in the assessment compared to when experimental values were used. As a consequence PEC values covered a wider range were give a more conservative assessment which resulted in more conservative risk estimates. The opposite result was obtained for the comparison of QSAR-based and experimental-based effect assessments. Experimental values were available for two species and therefore a safety factor was used. When using a QSAR prediction, the assessed effect were less uncertain since it was based on three instead of just two species, and therefore less penalization for uncertainty was added (as safety factors). This resulted in a less conservative risk estimate. Our main conclusion is that non-testing versus testing based risk assessments are different, but this difference depends to a large extent on how uncertainty is dealt with. As long as the predictions are precise (i.e. with a good coverage of the experimental values) non-testing information is a useful complement to reduce uncertainty in existing testing information of effects.

This case-study did not consider uncertainty in tested physic-chemical properties, not because it does not exist, but because that uncertainty is not included in the data. The uncertainty in QSAR predictions were derived from predictive inference based on the information in the underlying QSAR data. Besides predictive uncertainty, the reliability in predictions was evaluated for the QSAR models based on what was known about the applicability domain.

Conclusions of the case study

This case-study exemplifies the regulatory consequences of using non-testing information in the absence of testing information, but can also be seen as illustrating the consequences of combining non-testing information with weak testing information as a weight-of-evidence approach. A small discrepancy between non-testing and testing based risk assessment may not only be an effect of either accurate or inaccurate QSAR predictions. For example, in case when the influence of a single parameter on the outcome of risk assessment is small in comparison to other parameters in the assessment, inclusion of either testing or non-testing information does not make a large difference on the risk calculated. The study shows that QSAR uncertainty needs to be put in perspective to other uncertainties. The exposure assessment of the PBDEs shows that the influence of QSPR predicted parameters is small in comparison to whether or not the photolytic rate constant for degradation in air is considered. In order to generalize the impact of non-testing information provided by QSARs in chemical risk assessment, the approach described here is to be done on a larger set of chemicals carefully selected to represent chemical space by experimental design. General conclusions on the reliability of non-testing information in risk assessment are difficult to make, since the importance of different sources of information depends on each other, the context for the assessment, and the decisions made.

<u>Case study 3 – Prioritization based on PBT evaluation on BDEs and uncertainty analysis</u>

In this case study, the individual PBT properties of BDEs were assessed on the basis of QSAR-derived endpoints and a relative simple system was established for integrating the individual PBT-scores. Subsequently, the relative PBT scores for each of the BDE congeners were calculated and the congers were ranked. In addition, predictive reliability was assessed as this is a measure of confidence of the prioritization ranking obtained and as it might flag congeners for which the confidence in predicted PBT score is unacceptable low. The case study is included in Appendix 3.

Summary of the case study

Ranking BDEs according to urgency of concern and urgency of further testing in order to increase the confidence in the ranking, was performed based on relative PBT scores derived from QSAR evaluated endpoints used as in the PTB classification under REACH. Relative PBT scores were assigned based on the rank of the compounds according to the P, B and T in an increasing order. A relative PBT score was assigned to be the product of these scores, i.e.

PBT score = rank {in ascending order} (P score * B score * T score).

Conclusions of the case study

The case study allowed assessing the BDE-congener of highest concern. Apart from focussing on compounds of high concern, risk managers seek to avoid missing to give high concern to a highly hazardous compound, i.e. to commit errors of type II. In this case study this would mean that risk managers would be careful about BDEs assigned a low relative PBT-score and a low predictive reliability, as this means that the relative PBT-score of these compounds may be underestimated.

Case study 4 – Prioritization based on hazard assessment of BTAZs

Summary of the case study

Hazards were assessed for the 386 compounds constituting the CADASTER list of (benzo)triazoles. The assessments was based on PNEC values, calculated from QSAR predictions of fish, algae and daphnia. QSAR predictions had been generated from a consensus QSAR developed within the CADASTER project and available via the CADASTER website. PNEC-values for each of the compounds were assessed as the minimum EC_{50} out of the three species with an uncertainty factor of 1000, according to common procedure in hazard assessment.

Conclusions of the case study

The main finding of the study is that relative Hazard increased with molecular weight (dots in Figure 5). Furthermore, the confidence in assessments was lower for the compounds for which the most extreme hazard was calculated (triangles in Figure 5). Once the domain of applicability of the QSAR models was properly defined, the final ranking of a list of 386 BTAZs on the basis of calculated hazards could be derived fairly easily. The possibility of performing multiple assessments based on one modelling framework speaks for the usefulness of QSAR integrated assessments. Figure 5 is an example of how to communicate outcomes from multiple assessment over a multivariate characterization of chemical space including lower quality in individual assessments. The case study, including the actual hazard ranking, is summarized in Appendix 4.

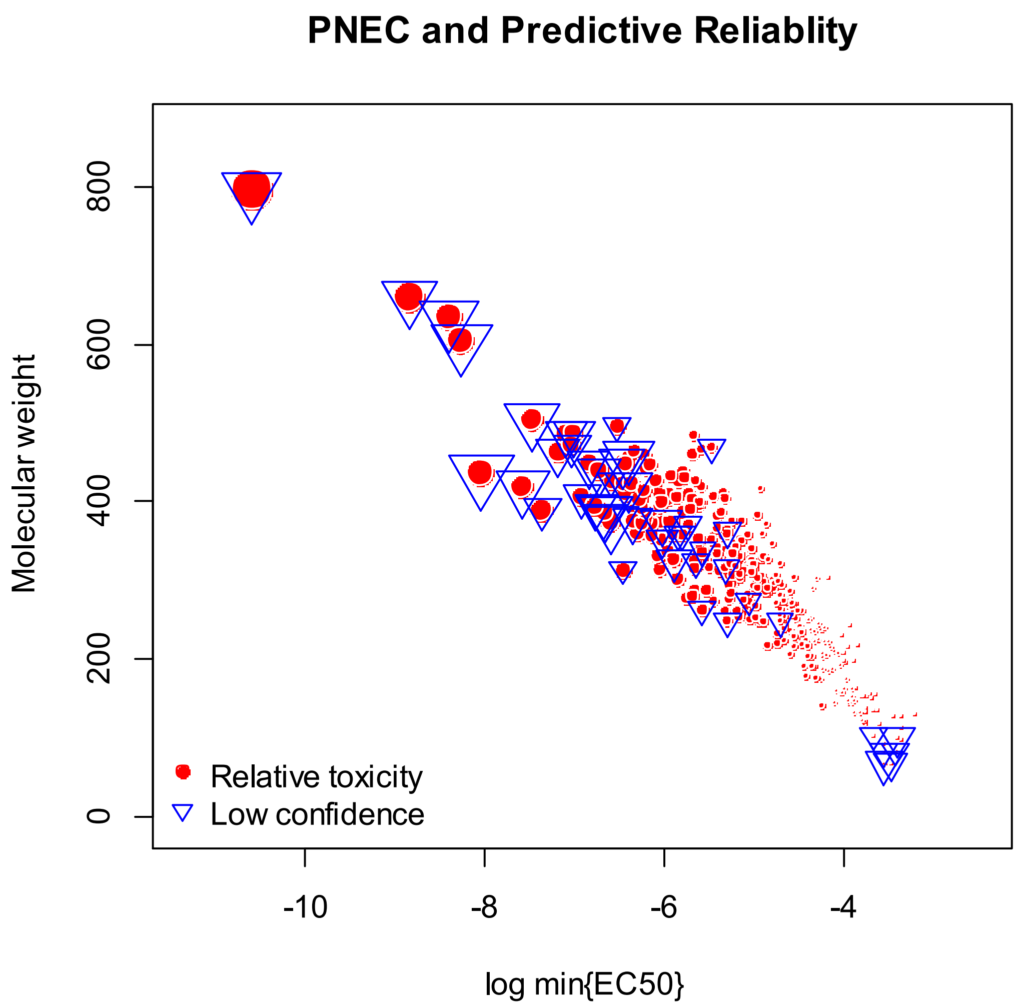

Figure 5. The spread of relative hazards and relative confidences in assessments stemming from individual QSAR predictions with low confidence evaluated by the applicability domain.

<u>Case study 5 – Prioritization based on risk assessment of BTAZs</u>

Summary of the case study

This case study describes a ranking based on risk assessments that are in turn based on a unit emission scenario. Predictive distributions for QSARs were used as sources in uncertainty analysis. The ranking of BTAZs was done based on assessment of PNEC and PEC given a unit emission. As PEC-values are proportional to emission, the ratio between PNEC/PEC (i.e. the reciprocal of the Risk Characterization ratio = PEC/PNEC) can be interpreted as the Maximum Permissible Emission (MPE).

The PEC and PNEC assessments were based on several QSARs to predict input parameters of physico-chemical properties or activities. Input parameters do more or less influence the final outcome of an assessment. Uncertainty in QSAR predicted input parameters was quantified as probability distributions describing the variation in a prediction that can be derived by statistical principles. These uncertainties had been propagated through the assessment by Monte Carlo simulation and contributed to the total uncertainty in the output (MPE). Sensitivity analysis showed differences in the relative contribution to uncertainty from different QSARs. The case study is given in Appendix 5.

Conclusions of the case study

The treatment of uncertainty described so far considers a quantitative uncertainty in a QSAR prediction, i.e. related to the magnitude of the error between having an experimentally based estimate and a QSAR prediction. Uncertainty in a QSAR prediction is also qualitative and depends for example on the degree of extrapolation. Given differences in the sensitivities of the final outputs to QSAR predicted input parameters, the influence of lower confidence in individual predictions may be both more or less relevant to the overall confidence in an assessment. In order to evaluate the influence of quality uncertainty in MPL we propagated the possible lower confidence in individual QSAR predictions, as seen when a compound is out of the applicability domain. In this case study a lower confidence due to a compound being out of the applicability domain was considered by enlarging the quantitative uncertainty in the corresponding QSAR prediction. Compounds were judged less reliable when their hat value exceeded the chosen cut-off. The difference in MPL based on the original assessment and an extended assessment including the enlarged quantitative uncertainty in the QSAR prediction showed the relative contribution of lower predictive reliability.

The QSAR-based descriptor informed MPL scheme was trained on 8 representative compounds using a PLS and was then applied on a list of selected 386 BTAZs. It was found that the assessment with low predictive reliability did not constitute extreme values. The case study thus exemplifies the inclusion of uncertainty analysis in QSAR prediction in assessment of the relative risk of compounds of similar chemical structure, falling within a specific chemical class of contaminants.

Case study 6 – The impact of uncertainty in QSAR integrated hazard assessment

Summary of the case study

A QSAR integrated hazard assessment was prepared for (Benzo)Triazoles (BTAZs) to illustrate QSAR integrated Chemical Safety Assessment as performed in the CADASTER project. A compound was classified as potentially toxic by comparing a derived hazardous concentration in aquatic environment to a predefined threshold. An assessment was based on QSAR predictions of aquatic toxicity on three species: algae, daphnia and fish. First we identified the lowest Effect Concentration where 50% of individuals of the most sensitive population (among those tested) are affected, i.e. EC50. Following the recommendations by REACH [2], we classified a compound as "very toxic" to the aquatic environment if the EC50 value on the most sensitive (evaluated) species, min{EC50}, was smaller than 1 mg/L.

QSAR predictions were derived for 386 BTAZs based on consensus modelling [1]. With the aim to illustrate the effect of considering uncertainty in QSAR predictions, we based hazard assessments on either QSAR prediction without uncertainty (i.e. point predictions) or with uncertainty (i.e. by a probability distribution for the error). The underlying QSARs predicted point predictions only. Uncertainty in QSAR predictions was characterized as a Normal distribution (symmetric bell shaped curve) with the point prediction as its mean and predictive error as its standard deviation. Predictive error was assigned the Root Mean Squared Error (RMSE) value derived for the training QSAR data sets. The RMSE was chosen as a good approximation of the average predictive error for compounds that are in the models domain of applicability. This approach to assess the predictive distribution in a QSAR prediction may be categorized as an expert judgment informed by statistical measures. All compounds were predicted by the three QSARs even though some of them fell out of one or more applicability domains. Compounds with the least reliable predictions were identified by the Maximum Absolute Difference (MAD) between individual predictions in the consensus modelling. Compounds with for which at least one MAD score among the three QSAR predictions were larger than 0.9 were judged as being assessed with low confidence.

The hazard assessment based on QSARs considered uncertainty in QSAR predictions either by uncertainty analysis or by using single values from the corresponding QSARs. This was meant to reflect conservative assessments and different levels of risk aversion. Uncertainty analyses were done by Monte Carlo simulation, where random samples were derived from the corresponding predictive distributions and, in each iteration, the min{EC50} value stored. The resulting uncertainty in aquatic toxicity (i.e. min{EC50} values) is described by a probability distribution. From this probability distribution we calculated the expected value, the median and the 5th percentile. These were used to illustrate the use of single values from the corresponding QSARs as input to the hazard assessment. A best guess or most likely value of aquatic toxicity can be provided by the corresponding medians and expected values. The median does not consider extreme values, while the expected value weights all possible values with their likelihood of occurring. Differences in expected and median values are found for skewed distributions with the presence of either high or low extreme events. When the interspecies variability in sensitivity is relatively larger than the uncertainty in individual QSAR predictions, the uncertainty in the min{EC50} value will be dominated by the uncertainty in the most sensitive species. In the QSAR models provided here these predictions have a symmetric distribution. When species interspecies variability is small in comparison to QSAR uncertainty, the minimum out of three values should result a skewed distribution. In this case-study the differences between median and expected values were negligible, meaning that the uncertainty in the classification variable was rather symmetric. The case study is given in Appendix 6.

Conclusions of the case study

Consideration of QSAR uncertainty resulted in more cautious classifications and an avoidance of making errors of type II. 19 out of 386 compounds were classified as toxic after QSAR uncertainty in input had been taken into account. Adding risk averse behaviour, an additional amount of 115 compounds were classified as potentially toxic.

We found that using conservative values for QSAR predictions (5th percentile) as input to the hazard assessment resulted in an increased probability of making error of type II compared to classifications based on the 5th percentile of the output.

To conclude, the impact on decision making from considering uncertainty in QSAR predictions was a reduced probability of making errors of type II. This effect was found when the full predictive distribution was used as input. Reducing the information on predictive uncertainty to a conservative value in the input to the hazard assessment did not automatically lead to more conservative classifications. Seven compounds were classified as non-toxic under the conservative assessment, while these compounds were classified as potentially toxic based on hazard assessment with probabilistic uncertainty analysis and risk adverse behaviour. Using conservative values to specify input both increase the probability of committing errors of type II, hinder the decision maker to be risk neutral, and force the decision maker to be risk averse to an unknown degree.

<u>Case study 7 – Uncertainty analysis in QSAR integrated risk assessment</u>

Summary of the case study

A non-safe emission occurs when the Environmental Concentration (EC), exceeds the No Effect Concentration (NEC). Fate and effect assessments were made to find the Predicted EC (PEC) and Predicted NEC (PNEC) of eight BTAZs. Uncertainty in the value of these quantities was expressed as probability distributions around the ratio between PNEC and PEC. When there is strong evidence that EC will be larger than NEC the decision is to apply risk management to reduce emissions, or refine the assessment to improve the evidence. We refer to these actions as “to regulate”. The case-study used several QSARs to assess PEC and PNEC for risk assessment. The purpose of the cases-study was to demonstrate the integration of QSARs into probabilistic risk assessment and was therefore based on a hypothetical common emission scenario for all evaluated compounds and did not consider all relevant non-QSAR sources of uncertainty.

The case-study was based on the QSAR integrated risk assessment developed within CADASTER. Fate assessments (PEC) were made using Simplebox under a unit emission rate. Effect assessments derived PNEC as the minimum out of QSAR predicted EC50 values on three aquatic species divided by an assessment factor of 1000. The level of emission was here adjusted to 500 kg/day in order to obtain compounds classified as safe and as risk. Further details of the case study are given in Appendix 7.

Conclusions of the case study

Assessment was done on two levels of complexity in the consideration of uncertainty. The output from a deterministic risk assessment (tier 0), i.e. where uncertainty is not considered, results in most likely values on PEC and PNEC. Here all compounds investigated had a Risk Characterization Ratio (RCR) below one which would indicate that they are safe. Seeking to avoid making erroneous risk classifications, probabilistic assessments were performed to quantify uncertainty (tier 3). The probabilistic evaluation of risk showed that all compounds were still safe since the probability of PEC exceeding PNEC was less than 5%. Considering sources of uncertainty does in general lead to safer decisions, and in that respect is uncertainty from QSAR predictions no exception.

Two of the compounds investigated were judged as being on the borderline of at least one QSAR model used as input to the assessment. To evaluate the influence of these QSAR predictions with lower confidence, we made a reassessment of risk where the corresponding predictive distributions were enlarged by an arbitrary factor (here the standard deviation in the predictive distribution had been multiplied by 10). This resulted in an enlarged risk. This sensitivity analysis aimed towards the influence of low confidence in individual QSAR predictions showed that risk classification of one of the compounds was sensitive to the lower confidence from QSAR predictions, as the 95th percentile of the RCR changed from being less than, to larger than one. In this case, the assessment of this compound may need to use other sources of background information to hold the same quality as the others, since we still may be unsure whether the risk is assessed as acceptable or not.

Overall, the case study exemplifies how to deal with uncertainty at different tiers of assessment. In addition it is to be concluded that inclusion of alternatives to testing (in this case QSAR estimates of toxicity) needs to be done with care: in all cases additional verification of the outcome of the risk assessment needs to be performed.

Case study 8 – Prioritization based on hazard assessment of PFCs

Summary of the case study

The use of QSAR-derived Species Sensitivity Distributions was exemplified, including consideration of the possible lack of sufficient species information to optimally support this approach. The SSD method is to fit a Species Sensitivity Distribution to log EC50 values and subsequently derive the hazardous concentration considering uncertainty from sample size. Uncertainty in the hazardous concentration based on EC50 values as point predictions were derived as the non-central Student-t distribution. The PNEC value is then the median of the distribution for the Hazardous Concentration (HC). Uncertainty in QSAR predictions were considered by performing an outer loop using Monte Carlo simulation to sample from the predictive distributions and storing the median HC value in each iteration. PNEC was derived by dividing the Hazardous Concentration by a factor of 1000 to open up for a comparison to the Assessment Factor method. A point value of the PNEC was taken as the median (Alt C) or the 5th percentile (Alt D) of the distribution for the Hazardous Concentration.

Conclusions of the case study

It first is concluded that when ranking without considering quality of available information, there is no need to derive the AF since we only make relative comparisons and the AF is equal for all compounds. The need to consider AF is introduced when the PNEC is derived for the purpose of risk assessment to be compared to a threshold for classification or another quantity such as the Predicted Environmental Concentration.

The list of 93 PFCs were ranked according to the relative hazard calculated as –log PNEC and the most conservative way to assess the minimum value of EC50. It was found that the confidence in assessments that can be understood from the statistical model was dependent on the length of the number of carbon atoms of the PFCs. Since the relative hazard was based on min(EC50) and molecular weight, the lower predictive reliability appeared on difference places in the ranked list. The compounds identified as of most concern are assessed with high confidence. Compounds on the lower part of the list and with low confidence may not be acceptable assessments.

Case study 9 – QSAR integrated fate assessment of PFCs

The Role of Uncertain K_{oc} Predictions in the Overall Persistency and Long Range Transport Potential of Perfluorinated Chemicals.

Summary of the case study

Perfluorinated chemicals (PFCs) are wanted chemicals in products to resist grease, oil, stains, and water, because of their combination of hydrophilic and lipophilic properties. For an assessment of the potential risks, one needs to quantify their environmental persistency, potential for bioaccumulation, and potential toxicity. When empirical data are lacking, substance properties can be predicted. The goal of this study was to assess the influence of uncertainty in the QSAR prediction for the organic carbon-water partition coefficient on the fate assessment of perfluorinated chemicals. The multimedia fate model Simplebox was used to estimate environmental fate in terms of overall persistency (Pov in days) and long range transport potential (LRTP, dimensionless). Parameter uncertainties were treated as probability distributions, and propagated by Monte Carlo simulations. The current modelling framework was demonstrated on 4 PFCS. Depending on the emission compartment, typical values for Pov ranged between 286 and 302 days for FTOH (8:2), between 549 and 608 days for FOSA, between 700 and 799 days for Perfluorohexanesulphonyl fluoride, and between 482 and 546 days for N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide. We found that uncertainty in Pov ranged from 8 percent of the typical value to 63 percent of the typical value. The LRTP was high (>0.95). Uncertainty in typical values for the LRTP was small: maximum 1.7 percent of the typical value when PFCs were emitted to soil. The current results should be interpreted cautiously, since dissociation was not taken into account and the QSAR was not validated due to data limitations.

Conclusions of the case study

We conclude that QSAR uncertainty in the organic carbon-water partitioning coefficient of PFCs has only small influence on the uncertainty in the potential for long range transport, but can have substantial influence on the uncertainty in the overall persistence. The case study thus clearly illustrates that uncertainties and variability in input parameters in the fate model will have different impacts on the variability in the output of the model for the various fate parameters. The impact of variance in input in variance in output is dependent on the type and magnitude of uncertainty and of the actual model settings, and hence is compound-specific.

4 Implementation of probabilistic risk assessment as a webtool

One of the major goals of the CADASTER project was to provide a wide dissemination of project results by means of the CADASTER web site http://www.cadaster.eu and the QSPR Thesaurus http://www.qspr-thesaurus.eu database. As part of these activities, the “Risk Assessment” webtool http://www.qspr-thesaurus.eu/risk has been developed to demonstrate the contribution of uncertainty in experimental and QSAR predicted values on Fate, Effect and Risk assessment. It is implemented within the QSPR-THESAURUS web site http://www.qspr-thesaurus.eu of the CADASTER project, which is based on the On-Line Chemical Modeling Environment (OCHEM) developed by the HMGU group. The latter system was extended to include tools and features required for the CADASTER project. The detailed technical description of the developed webtool is available in Appendix 10.

Case study: Prioritization based on risk assessment for PBDE using CADASTER webtool (see also Appendix 11)

The main purpose of this case study was to exemplify the use of the developed webtool to demonstrate the influence of uncertainty in the calculated physico-chemical and toxicity values on the probabilistic risk assessment of compounds. This case study thus extended the case studies with a few PBDEs from Chapter 3 by extending them to all PBDEs available in the QSPR Thesaurus web.

Summary of the case study:

Exposure (PEC) and effect (PNEC) were assessed based on QSPR and QSAR predictions for PBDEs. We revised models from the previous study with respect to their reproducibility using the available workflow. The models based on 2D descriptors could be easily reproduced. The models based on 3D descriptors, and/or those not available in QSPR Thesaurus, could not be reproduced due to differences in the structure optimised protocols. These models were redeveloped using 2D descriptors and protocols implemented in QSPR Thesaurus, and were shown to provide similar prediction accuracy. The implemented and re-developed QSAR models were used to provide the risk assessment of PBDEs.

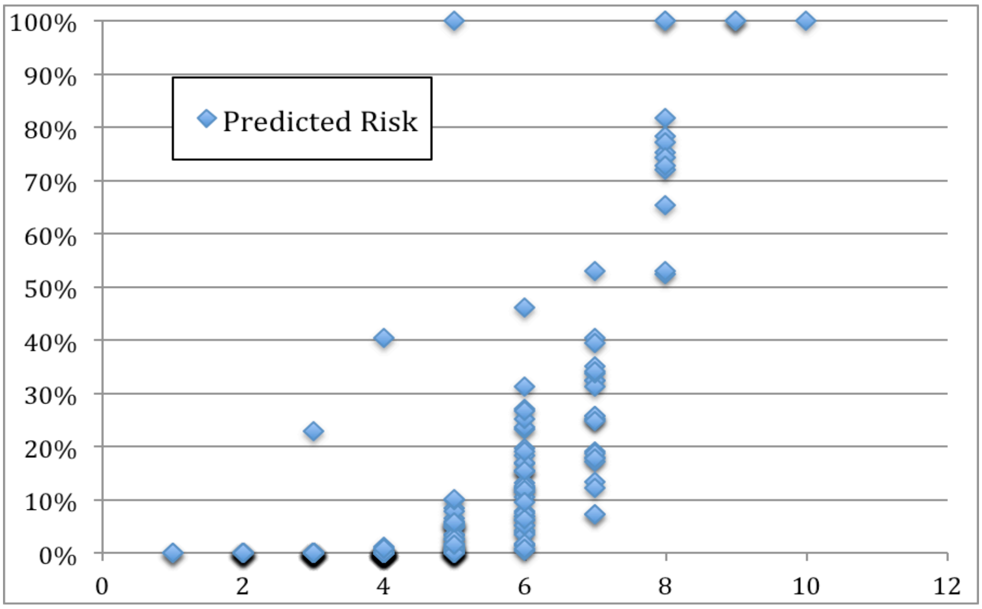

Figure 6. Environmental risk for PBDE as function of the number of Br atoms in the molecule..

Figure 6 shows that PBDE congeners with more than 5 atoms represent a considerable risk for the environment. However, PBDE phenols were predicted to be more toxics compared to PBDE congeners with the same number of Br atoms. The developed webtool allows to make such analysis easily and process large number of molecules. Moreover, all intermediate results of calculations can be downloaded and be made accessible for the review by regulators or interested parties.

Conclusion: We have developed a web tool for the exemplification of the use of QSAR models for the fate, effect and risk assessment of chemical compounds. The developed webtool allows an easy and interactive analysis of the fate and effect assessment of chemical compounds using Simple Box and the SSD approach, respectively. The PEC and PNEC values calculated with both these approaches can be used to provide a risk assessment of chemicals. We have also demonstrated its use for the risk assessment of 207 PBDE congeners.

5 Conclusions and recommendations

The case studies described in chapter 3 and the implementation of alternatives in probabilistic risk assessment as operationalized within the CADASTER website (chapter 4) with the focus on quantifying uncertainty and variability, have signalled first of all the need of a general conceptual framework for probabilistic risk assessment. This conceptual framework is needed to define and understand uncertainty in a QSAR prediction in a decision context and to provide a framework for the theoretical understanding of approaches to assess uncertainty in predictions covering probability models and principles for statistical inference. The focus within CADASTER in this respect has been on applying the conceptual framework for predictions for QSAR regressions (i.e. with a continuous as opposed to a categorical response) which is common within environmental risk assessments and for which uncertainty is less often reported probabilistically. The basic framework allows to propagate uncertainty into the final risk estimates. Environmental risks are typically estimated in a deterministic way using single point estimates for both exposure (PEC) and effects (PNEC). For uncertainty analysis these point estimates (PEC and PNEC) are replaced in the framework by probability distributions. Uncertainty in PEC is derived by Monte Carlo simulation of the underlying fate model (which is Simplebox in the case of REACH) based on the specified uncertainty in input parameters. PNECs are recommended to be derived as a Species Sensitivity Distribution (SSD) in order to capture the variability between species in the ecological system. The probabilistic PEC and PNEC are subsequently combined into a quantitative risk measure. The deterministic measure Risk Characterization Ratio (RCR) is hampered by not being a measure of risk - it is sensitive to scaling and is therefore not comparable between substances. Instead, the probabilistic measure of risk of the probability of an undesired effect P(PEC > PNEC) is used. This probability can alternatively be expressed as the Expected Risk (as illustrated in Figure 6 for the case of BDE-28: this figure is also included in Appendix 2), which is the expected fraction of species affected for an uncertain exposure. As a matter of course it is realised that in case of lack of sufficient effect data/models it will not be possible to generate an SSD. In that case common alternatives like standard safety factors that depend upon the number and type (for instance chronic versus acute data or model predictions) of effect data or model predictions are used in the framework, as commonly accepted in current risk assessment.

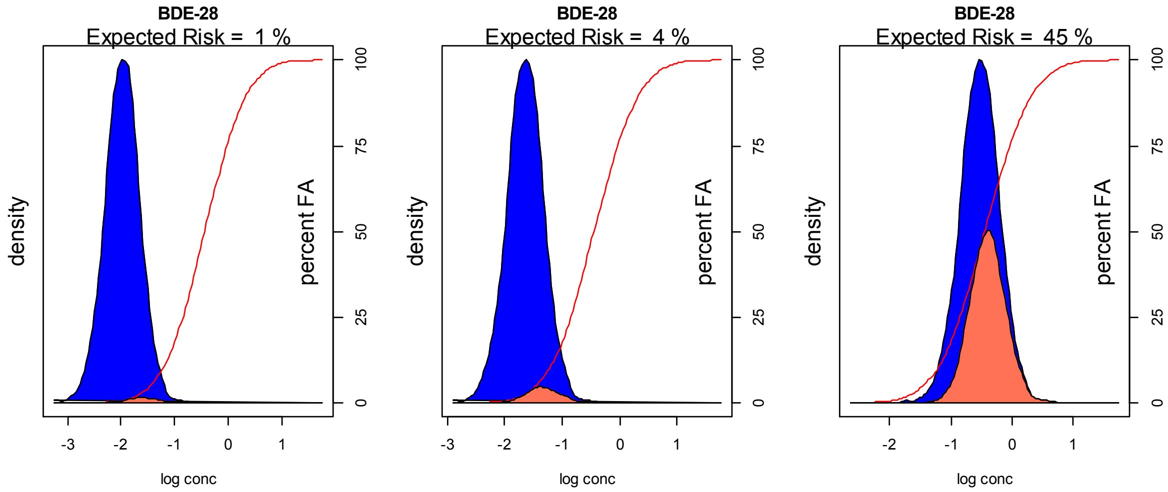

Figure 6. Illustration of the conceptual framework based on calculating expected risk as a measure of the extent in which PEC and PNEC distributions overlap.

In line with the issues raised in chapter 2 as related to the implementation of Integrated Testing Strategies (ITS) in risk assessment, the general risk assessment framework of quantifying the extent in which PEC and PNEC distributions overlap as exemplified in Figure 5 allows for a well-balanced integration of experimental data and data obtained by means of testing alternatives in a viable management strategy. Thereupon, the framework allows for quantifying the predictive distribution of parameter estimates, as for the case of QSAR estimates denoted in the workflow for QSAR based risk assessment shown in Figure 4 of chapter 2.

The following elements are recommended to be integrated in the management strategy for assessing hazards and risks of groups of structurally related compounds:

- Operationalization of the constituents that are suited building stones for Integrated Testing Strategies, like *in silico* methods, read across, exposure based waiving, and experimental test results for chemicals within the specific class of compounds. Within the CADASTER project this element was operationalized by designing, filling, and expanding the database on experimental data, models, and other alternatives.
- Performance of reliability check of the data generated for specific compounds, including quantification of the associated uncertainties. In principle most confidence is put into experimental data, but one of the criteria in assessment of the reliability of data, may be to perform cross checking of the data by comparison with model predictions for chemicals within the chemical domain of the models. Vice versa, this procedure allows for external validation of the models, thus increasing the confidence in the models.

- Integration of experimental data and data obtained by means of alternative methods into a probabilistic distribution of the endpoints needed for environmental risk assessment. This implies that an explicit uncertainty analysis should be performed on both the experimental data (often point estimates) and the model predictions (often a probability distribution for the error and a qualitative statement of the confidence in a prediction). Key to integrated probabilistic risk assessment according to the philosophy of REACH is consensus building and weight-of-evidence approaches that integrate all possible types of information in deriving the best estimate. A specific approach to consensus building was demonstrated within CADASTER by combining predictions of a number of independently developed models into a consensus model which was validated by means of independently generated experimental data. On top of this, the case study on non-testing versus testing based risk assessment of PBDEs (case study 2) showed that discrepancies in the outcome of risk assessment may be observed as dependent on the use of either experimental or model data. Often – but not always – supplementing limited information from experimental testing that in itself results in an conservative risk estimate, by applying a weight-of-evidence approach in which alternative approaches are incorporated will result in less conservative estimates. In itself, a discrepancy between testing and non-testing based risk is an indication that further efforts are needed to supplement the constituents of the weight-of-evidence approach.
- Calculation of PEC and PNEC and the underlying probabilistic distributions given the uncertainties in the input parameters for both PEC and PNEC. This will substitute for single point estimates typically used to express exposure and effects. As stated above, uncertainty in PEC is for instance derived by Monte Carlo simulation of the underlying fate model based on the specified uncertainty in input parameters, and uncertainty in PNECs are recommended to be derived as QSAR-integrated SSDs.
- Combine probabilistic PEC and PNEC are into a quantitative risk measure, either the deterministic Risk Characterization Ratio or, preferably, the probabilistic measure of expected risk of the probability of PEC exceeding PNEC.

In general it is to be concluded that the integration of alternatives into probabilistic risk assessment is possible given proper assessments of (predictive) uncertainty and (predictive) reliability to inform the characterization of parameter and model uncertainty. Treatment of uncertainty is a key issue that in turn is context dependent, and should be interpreted in relation to the background information available. Integration of QSARs in chemical safety assessment is an example where it is obvious that aspects of uncertainty are linked to the background knowledge, since we have the option to improve background knowledge by further testing.

Furthermore, probabilistic risk assessment is supported by weight-of-evidence approaches and predictive models that are partly derived from Bayesian predictive inference. Predicting must be done with care, and the use of different bases for predictive inference is possible when a model is treated as a scientific based hypothesis supported by empirical data.

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APPENDICES

Appendix 1

Case study 1: Uncertainties in triazole risk assessment based on QSARs

Introduction

Triazoles are chemical compounds that are globally used for fungi control. Their importance for pest management has increased, among other reasons because of their broad spectrum of activity (Klix, Verreet et al. 2007). Belonging to the group of demethylation inhibitors (DMIs), triazoles act specifically on the biosynthesis of ergosterol (Maštovská 2005). Each triazole compound may act slightly different on the ergosterol production pathway, but in the end they all cause abnormal fungal growth and death. The application of triazoles to plants and crops can lead to contamination of the aquatic environment, i.e. ground and surface waters (Li and Randak 2009). Although triazoles were designed to interfere with the ergosterol biosynthesis in target fungi, they can also display different modes of action in non-target organisms. Hassold and Backhaus (2009) showed that DMI fungicides from four different chemical classes, including triazoles, exhibit baseline toxicity as well as specific toxicity in *Daphnia Magna*. Furthermore, Ankley et al. (2005) showed multiple modes of actions of the DMI fungicides prochloraz and fenarimol in Fathead Minnow. Hassold and Backhaus (2009) emphasize the risk for aquatic invertebrates due to the high toxicity and ubiquitous use and resulting occurrence in the aquatic environment of demethylation inhibitors.

For proper risk assessment, a systematic procedure through estimation of exposure and effects is required (Van de Meent 1998). Important steps are the determination of the Predicted Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC). However, the risk assessment of triazoles is hampered by the fact that chemical monitoring data as well as toxicity measurement data are rarely available. Therefore, they are one of the four classes of chemicals studied in the European Union-Framework Project-7 CADASTER (CAse studies on the Development and Application of in Silico Techniques for Environmental hazard and Risk assessment) project (EU FP7 CADASTER, 2009), in which the authors are involved. Within this project, quantitative structure-property relationships (QSPRs) have been developed that can be used to model a chemical's fate in the environment when measured data for chemical properties are lacking. Similarly, quantitative structure-activity relationships (QSARs) have been developed to predict a chemical's effects.

The use of QSARs and QSPRs makes a full risk assessment possible, provided that their predictions qualify as replacement of experimental data or empirical observations. However, with respect to the reliability and uncertainty of the input data, careful interpretation of the outcome is required. Therefore, the use of QS(A)PRs in risk assessment can be justified if their uncertainties were treated properly and do not have substantial impact on the regulatory decision following risk assessment. The goal of this study was to determine the influence of the use of QS(A)PRs on the uncertainty in the outcome of a risk assessment for triazoles. We implemented QSPRs in the multimedia fate model Simplebox (Den Hollander, Van Eijkeren et al. 2004) to predict the aquatic concentration of triazoles after a single unit emission, and used QSARs to model the no effect concentrations. In the end, the uncertainty in the outcome was quantified, and a sensitivity analysis was performed to determine the relative contribution of the different predictive models to the overall uncertainty.

Methods

Risk assessment based on QS(A)PRs

The environmental fate of the triazole fungicides is determined by different chemical properties and processes. We used QSPRs to predict chemical properties, which enabled the environmental fate modeling of the triazoles. Soil sorption partition coefficients (K_{oc}) were predicted with a multiple linear regression developed for a set of heterogeneous, organic, non-ionic compounds by Gramatica et al. (2007). Aqueous solubility, melting point, and vapor pressure were predicted with the multiple linear regressions of Bhhatarai et al. (2011). The rate constants for hydroxyl radical reaction in air were predicted with the multiple linear regression of Roy et al. (2011). All QSPR models fulfill the fundamental principles laid down by the OECD (OECD 2007). The descriptors used in the QSPR models can be found in Table 1. For more information about the descriptors used in the multiple linear regressions we refer to the references mentioned.

Biodegradation rates are a function of both the chemical properties and the surrounding environment. They are very uncertain and have not been measured for most chemicals. The time required for biodegradation in the aquatic environment was predicted with combined use of the Biowin3 semi-quantitative ratings from Episuite™ (Boethling, Howard et al. 1994) and the experimental half-lives determined by Aronson et al. (2006). The half-lives for soil and sediment were assumed to be two and nine times as long as in water (US EPA 2002).

The predicted environmental concentrations of triazoles in fresh water were modeled with the Simplebox model (Den Hollander, Van Eijkeren et al. 2004). This is a fugacity model in which the environment is modeled as a set of homogenous compartments; one compartment for each environmental medium in which the chemical is assumed to be evenly distributed. Results from Simplebox are commonly used in EU risk assessments for new and existing chemicals (European Commission 2003). We modeled concentrations on the regional, continental and Northern hemispheric scale, after a single unit emission to agricultural soil.

Simplebox was also used to predict the triazoles' potential for long-range transport (LRTP) through the environment. It was defined as the fraction transferred from the regional scale to the continental and Northern hemispheric scale:

$$LRTP_x = M_{r,x} / M_{tot,x} \tag{1}$$

In this equation $LRTP_x$ is the dimensionless long-range transport potential of chemical x, $M_{r,x}$ is the steady state mass of chemical x on the regional-scale, $M_{tot,x}$ is the total steady state mass of chemical x present in the environment.

Long-range transport potential and degradation half-lives together determine the chemical's overall persistence. A commonly used numerical indicator for the overall persistence of a chemical is its overall residence time in the environment (Klasmeier, Matthies et al. 2006). This can be calculated by:

$$P_{ov,x} = M_{tot,x} / E_x \tag{2}$$

where $P_{ov,x}$ is the overall residence time of chemical x in the environment (days), $M_{tot,x}$ is the total steady state mass of chemical x present in the environment (kg), and E_x is the emission rate of chemical x (kg/day).

According to the European Commission (2003), an aquatic effect assessment should be composed of at least one short term LC50 or EC50 for each trophic level, i.e. a base set of algae, Daphnia and fish. In this study, we used QSARs based on dragon descriptors for the derivation of toxic concentrations. Three multiple linear regressions were available for triazoles, namely for the LC50 of *Onchorynchus Mykiss*, for the EC50 of *Daphnia Magna*, and for the EC50 of *Pseudokirchneriella Subcapitata*. The descriptors used in the QSAR models can be found in Table 1. With little effect data, the PNEC is determined by using fixed assessment factors that are calculated by means of a statistical extrapolation model with an arbitrary cut-off value set at a protection level of 95 percent of the species (Bro-Rasmussen 1988; European Commission 2003). This should account for, among other things, the different sensitivities of other untested species. Here, the PNEC was predicted as:

$$PNEC_x = \frac{\min(LC50_{s1,x}, EC50_{s2,x}, EC50_{s3,x})}{1000} \tag{3}$$

where the PNEC of chemical x (g/L) is the minimum of the toxicity measures available for chemical x in species 1 to 3 (*Onchorynchus Mykiss, Daphnia Magna, and Pseudokirchneriella Subcapitata*, respectively), divided by the assessment factor 1000.

The risk assessment was performed on the basis of the maximum permissible emission for chemical x (MPE_x in kg/day), i.e. the maximum emission to agricultural soil without effects in 95 percent of the aquatic species. It was calculated as the ratio of the PNEC and the aquatic PEC multiplied by the emission mass (E_x in kg/day):

$$MPE_x = \frac{PNEC_x}{PEC_x} \cdot E_x \qquad (4)$$

A risk assessment based on QS(A)PRs was performed for a selection of five triazoles: Tebuconazole (CAS 107534-96-3), Triazamate (CAS 112143-82-5), Bromuconazole (CAS 116255-48-2), Difenoconazole (CAS 119446-68-3), and Metconazole (CAS 125116-23-6). All were known to be commonly used.

Table 1: QSPRs used for the estimation of the physico-chemical properties at 25°C, and QSARs used for the estimation of the no-effect concentration

Parameter	**Unit**	**Multiple linear regression**	**Reference**
soil sorption partition coefficient (K_{oc})	L/kg	Log K_{oc} = – 1.92 + 2.07 VED1 – nHAcc – 0.31 MAXDP – 0.39 CIC0	Gramatica et al. 2007
aqueous solubility (WS)	mg/L	Log WS = 13.80 – 2.41·CICO – 0.44·AMW + 1.65·MATS7e	Bhhatarai et al. 2011
melting point (MP)	°C	MP = 1098.25 – 162.83·R2e + 53.22·GGI4 + 26.82·F03[N-N] – 1693.0·χ1A	Bhhatarai et al. 2011
vapor pressure (VP)	mmHg	Log VP = 17.30 – 15.67·BELp2 + 0.44·RBN + 1.38·B09[N-Cl]	Bhhatarai et al. 2011
rate constants for hydroxyl radical reaction (k_{OH})	cm^3s^{-1}/mol	Log 1/k_{OH} = 4.07 – 0.72 HOMO + 0.37 nX + 0.16nCbH – 0.34IDE	Roy et al. 2011
LC50 *Onchorynchus Mykiss*	Mol/L	Log 1/LC50 = -6.705 + 1.579 CIC1 + 13.251 Mp + 0.135 H-052 - 0.005 TPSA(Tot)	Cassani et al. 2013 (in review)
EC50 *Daphnia Magna*	Mol/L	Log 1/EC50 = 3.725 - 0.019 TPSA(NO) + 0.009 Aeigm + 0.048 nCar + 0.192 nHDon + 0.027 H-052	Cassani et al. 2013 (in review)
EC50 *Pseudokirchneriella Subcapitata*	Mol/L	Log 1/EC50 = 3.448 + 0.014 AEigZ + 0.09 T(N..S) + 0.15 Seigv	Gramatica et al. 2012

CIC0	Complementary Information Content index: neighborhood symmetry of 0-order (i.e. the degree of the diversity of the elements in the molecule)
AMW	Average Molecular Weight
MATS7e	Moran autocorrelation of lag 7 weighted by Sanderson electronegativity (i.e. the charge distribution)
R2e	R autocorrelation of lag 2 weighted by Sanderson electronegativity (i.e. the geometry topology and atomic weight assembly)
GGI4	topological charge index of order 4 (i.e. charge transfer between atom pairs)
F03[N-N]	Frequency of N - N at topological distance 3

χ1A	connectivity index of order 1 (Randic connectivity index) (which describes molecular branching and complexity)
BELp2	Lowest eigenvalue n. 2 of the Burden matrix weighted by atomic polarizabilities
RBN	number of rotatable bonds
B09[N-Cl]	Presence/absence of N - Cl at topological distance 9

VED1	eigenvector coefficient sum from distance matrix
nHAcc	number of acceptor atoms for H-bonds (N,O,F)
MAXDP	maximal electropological positive variation

HOMO	highest occupied molecular orbital
nX	number of unsubstituted sp^2-carbon in any ring, mainly aromatics
nCbH	number of unsubstituted sp^2-carbon in benzene-type rings
IDE	mean information content on the distance equality (topological descriptor similar to CIC0)
CIC1	Complementary Information Content index: neighborhood symmetry of 1-order (i.e. the degree of the diversity of the elements in the molecule)
Mp	mean atomic polarizability (scaled on Carbon atom)
H-052	H attached to C0(sp3) with 1X attached to next C
TPSA(Tot)	topological polar surface area using N,O,S,P polar contributions
TPSA(NO)	topological polar surface area using N,O polar contributions
Aeigm	Absolute eigenvalue sum from mass weighted distance matrix
nCar	number of aromatic sp^2-carbon
nHDon	number of donor atoms for H-bonds (N and O)

AEigZ	Absolute eigenvalue sum from Z weighted distance matrix (Barysz matrix)
T(N..S)	sum of topological distances between N..S
Seigv	Eigenvalue sum from van der Waals weighted distance matrix

Quantification of uncertainties

The integration of model predictions into probabilistic risk assessment requires validation of the QS(A)PR for its ability to make reliable predictions, and quantification of the associated uncertainty (ECHA 2008; Sahlin, Filipsson et al. 2011). Parameter uncertainty is treated as a probability distribution describing the range of possible values (or classes if categorical), and can be assessed using statistical methods based on empirical data or expert judgment. With respect to the use of QSA(P)Rs in risk assessment, model uncertainty means the reliability of a QSA(P)R in predicting a property of a specific compound (Sahlin, Filipsson et al. 2011). The reliability is among other things restrained by the applicability domain (AD) of the QS(A)PRs, which is a research area in development (Nikolova and Jaworska 2004, Gramatica 2007). We discussed the consequences of the AD for our risk assessment in the discussion section.

The experimental data underlying the multiple regressions were used to assess statistical uncertainty in the QSPR and QSAR predictions. The uncertainty in a prediction Y based on the descriptors W using a QS(A)PR as a linear regression fitted by ordinary least squares was assigned according to the approach of statistical inference discussed in Appendix 1 and 2. In this case, the predictive distribution is fully specified by the predictive mean PRED(Y), the predictive error SEP(Y), the number of data points in the training data set (n) and the number of descriptors in the linear regression model (p), as:

$$Y \sim PRED(Y) + t_{n-p-1} \cdot SEP(Y) \tag{5}$$

where t_{n-p-1} stands for the *t*-distribution with n–p–1 degree of freedom. The predictive error is estimated as

$$\left[SEP(Y)\right]^2 = \sigma^2 (1 + W^T (X^T X)^{-1} W) \tag{6}$$

where σ^2 is the variance in model errors and $(X^T X)^{-1}$ is the information matrix (Box and Tiao 1992).

Because of a lack of more precise information, the uncertainty in biodegradation half-lives was not treated with statistical methods. Instead we made an expert judgment and assigned a log-normal distribution (Slob 1994), of which the geometric mean and geometric standard deviation were based on the work of Aronson et al. (2006). This is an arbitrary but plausible choice since biodegradation shows natural variability.

Finally, the uncertainties in the predictive modeling output were determined in Monte Carlo Analyses using the spreadsheet-based application Chrystal Ball (Oracle©, Release 11.1.2.0.00, March 2010) in MS Excel with 10,000 iterations per run.

A sensitivity analysis was performed to determine the relative contribution of the uncertainty per input parameter to the uncertainty in the aquatic PEC, in the PNEC, and in the MPE for an emission to agricultural soil. Chrystal Ball was used to calculate the Spearman's rank correlation coefficients between each input parameter and the outcome variable, as a measure of statistical dependence between the two. By squaring the rank correlation coefficients and normalizing them to 100 percent, the contribution to variance was calculated. This way, the relative contributions were obtained for the impact a QS(A)PR has on the uncertainty in the outcome variable, via both its uncertainty and its model sensitivity.

Results

Probabilistic risk assessment

Figure 1 shows the median potential for long range transport of the five triazoles assessed in this study, which ranged from 2.64 $\cdot 10^{-5}$ for Difenoconazole to 3.27 $\cdot 10^{-3}$ for Tebuconazole, with 90% confidence intervals (90%-CIs) of up to six orders of magnitude. Looking at persistency, Triazamate differs from the other four triazoles. Its median value for overall persistency is 2.54 $\cdot 10^{1}$ days with a 90%-CI of almost three orders of magnitude, whereas the other chemicals have a median overall persistency of 1.46 $\cdot 10^{2}$ to 1.52 $\cdot 10^{2}$ days with accompanying 90%-CIs ranging two orders of magnitude. The differences between the five triazoles for the aquatic PEC show the same pattern as LRTP. The median aquatic PEC value after an emission of 1 kg/day to agricultural soil was the lowest for Difenoconazole (1.24 $\cdot 10^{-13}$ g/L), and the highest for Tebuconazole (1.41 $\cdot 10^{-11}$ g/L). The 90%-CIs ranged up to five orders of magnitude. Bromuconazole was the least toxic triazole in this study. We found median PNEC values ranging from 3.13 $\cdot 10^{-7}$ g/L to 2.27 $\cdot 10^{-6}$ g/L with 90%-CIs ranging one to two orders of magnitude. Consequently, the typical maximum permissible emissions to agricultural soil were highest for Bromuconazole and Difenoconazole, to be exact 2.09 $\cdot 10^{6}$ and 2.26 $\cdot 10^{6}$ kg/day, respectively, with 90%-CIs of four orders of magnitude. For Tebuconazole, Triazamate, and Metconazole we found lower typical MPEs, that is between 5.15 $\cdot 10^{4}$ and 8.00 $\cdot 10^{4}$ kg/day, with 90%-CIs ranging three to five orders of magnitude. **Table S1** (supporting information) shows the predictions of the input parameters with their predictive error (or geometric mean and standard deviation for the half-lives in water) for all triazoles in this study and the assigned distribution.

Sensitivity analysis

In a sensitivity analysis, the relative contribution of the uncertainty per input parameter to the variance of the outcome variable for an emission to agricultural soil was quantified. **Table 2** shows that the uncertainty in the aquatic PEC and MPE for agricultural soil was mainly determined by uncertainty in the soil sorption partition coefficient, and in the biodegradation rate in water. However, uncertainty in the toxicity to different species was also relevant. The contribution to variance was <0.05 percent for water solubility, melting point, vapor pressure, and hydroxyl radical reaction in air, which were therefore excluded from the table. The five triazoles in this study showed differences in the importance of the input parameters. The relative contributions to the variance of the MPE for agricultural soil ranged from 10.8 to 58.3 percent for the K_{oc}, from 30.1 to 82.7 percent for the $k_{biodeg,water}$, from 1.5 to 6.4 percent for the LC50 of *Onchorynchus Mykiss*, from <0.05 to 0.6 percent for the EC50 of *Daphnia Magna*, and from 1.9 to 10.6 percent for the EC50 of *Pseudokirchneriella Subcapitata*.

Table 2: Relative contribution to the variance of the aquatic PEC, of the PNEC, and of the maximum permissible emission to agricultural soil.

Deterministic parameter		Tebuconazole	Triazamate	Bromuconazole	Difenoconazole	Metconazole
Relative contribution to the uncertainty in the aquatic PEC (%)						
Physicochemical properties	K_{oc}	47.7	11.5	61.3	65.8	49.5
Biodegradation in water	$k_{biodeg,water}$	52.2	88.4	38.6	34.0	50.4
Relative contribution to the uncertainty in the PNEC (%)						
LC50 O. Mykiss		27.7	75.3	68.5	29.1	16.5
EC50 D. Magna		3.2	-	1.4	2.7	4.0
EC50 P.Subcapitata		69.1	24.6	30.1	72.2	79.7
Relative contribution to the uncertainty in the maximum permissible emission to agricultural soil (%)						
Physicochemical properties	K_{oc}	42.3	10.8	55.4	58.3	43.4
Biodegradation in water	$k_{biodeg,water}$	45.7	82.7	34.6	30.1	43.8
LC50 O. Mykiss		2.6	4.5	6.4	2.7	1.5
EC50 D. Magna		0.5	-	0.2	0.4	0.6
EC50 P.Subcapitata		8.8	1.9	3.3	8.4	10.6

Discussion

Methods

The environmental fate of triazoles in air was diminished by photolytic degradation via indirect photolysis, i.e. through a reaction with photo-oxidizing OH-radicals (European Commission 2003). In soil, indirect photolysis was not taken into account, since Kim et al. reported it is minimal (2002). In water, photolytic degradation involves both indirect photolysis and direct reactions according to some authors (Vialaton, Pilichowski et al. 2001; Vialaton and Richard 2002). Wallace et al. (2010) found as well that indirect photolysis via a reaction with photo-oxidizing nitrate-radicals significantly enhances the degradation of propiconazole in water. However, the relevance of direct photolysis, by UV irradiation, is not conclusive. Wallace et al. (2010) stated that propiconazole is stable to direct photolysis. Abramovitch et al. (2001) and Da Silva et al. (2001) explained that direct photolysis in water is not expected because triazoles do not absorb irradiation with a wavelength of $\lambda > 200$ nm. In addition, Breedveld et al. (2002) reported that direct photolysis in water requires high radiation doses. The European Commission (2003) concluded that direct photolysis is not significant. Hydrolysis in water was not included in the model calculations, since the European Food Safety Authority (2010) reported it is negligible for 1,2,4-triazole.

The applicability domains of the QS(A)PRs restrain their reliability, meaning that only the predictions that fall within the AD can be considered reliable. An informative review about the validation of QSARs was written by Gramatica (2007). She states that when the leverage value of a compound is lower than the critical value (which is depending on number of model variables and the number of the objects used to calculate the model), the probability of accordance between predicted and actual values is as high as that for the training set chemicals. **Table S2** (Appendix 4) shows that most QS(A)PR predictions were within the AD, except for the water solubility prediction for Triazamate, and the hydroxyl radical reaction rate in air for Difenoconazole. For Bromuconazole, the k_{OH} prediction was just outside the border of the AD. Whether the triazoles of this study are within the applicability domain of the QS(A)PR models does not directly influence the predictions themselves, but a prediction that is outside the model's AD has a higher uncertainty than what was calculated in this study. As stated by Nikolova and Jaworska (2003), it is a warning for model applicability, but not a final decision on prediction quality. In principle, there are two options. One could still judge that the QS(A)PR model gives a reliable outcome. This requires a decision on how to treat the extra uncertainty that is caused by being outside the AD, which is a research area that is still in development. A (hypothesized) mechanistic understanding of the modelled property could be a start to decide the best treatment for the extra uncertainty (Nikolova and Jaworska 2003). Furthermore, one could judge that the outcome of the QS(A)PR model is not reliable and cannot be used. In that case, either a better QS(A)PR or experimental data are required. Despite of one water solubility prediction and two k_{OH} predictions outside the AD, we think this risk assessment performed in this study is reliable. After all, the results of the sensitivity analysis showed the uncertainty in these parameters has negligible influence on the uncertainty of the outcome variables.

The PNEC calculations were based on a fixed assessment factor of 1000, because only a small dataset of acute toxicity predictions was available. The assessment factor should be applied on the lowest L(E)C50 value. It accounts for the intra- and inter-species variations; intra- and inter-laboratory variation of toxicity data; short-term to long-term toxicity extrapolation; and laboratory data to field impact extrapolation (e.g. multi-substance effects)(European Commission 2003). Since the assessment factor accounts for the uncertainty inherent in acute toxicity data (i.e. intra- and inter-species variations), and we also applied a Monte Carlo simulation to include the uncertainty in the QSAR model predictions, the calculated PNEC may be in this case be interpreted as a worst-case value. However, in case of one short-term L(E)C50 from each of three trophic levels of the baseset, variation from a factor of 1000 should not be regarded as normal and should be fully supported by accompanying evidence (European Commission 2003).

Figure 1: (a) Dimensionless LRTP, (b) Persistency in days, (c) aquatic PEC in g/L, (d) PNEC in g/L, and (e) MPE in kg/day, for an emission to agricultural soil of Tebuconazole, Triazamate, Bromuconazole, Difenoconazole, and Metconazole.

Interpretation of results

The uncertainties in the soil sorption partition coefficient, biodegradation rate in water, and toxic concentrations contributed to the uncertainty in the MPE for an emission to agricultural soil. The predicted K_{oc} of Triazamate is the lowest in this study ($5.87 \cdot 10^{1}$) with a relative contribution to variance of the MPE of 11 percent, whereas the other four triazoles have a higer K_{oc} ($>5.70 \cdot 10^{2}$) and a higher relative contribution to variance of the MPE (>43 percent). The K_{oc} is an important property of triazoles, because the high sorption to soil organic matter is probably responsible for the limited movement and leaching from the soil (Kim, Beaudette et al. 2002). Moreover, sorption to soil organic matter could also explain the moderate soil longevity, since it makes the chemical less available for micro-organisms to degrade. Nevertheless, soil microorganisms degrade triazole fungicides in soil, as reported for ipconazole by Eizuka et al. (2003). In accordance with that, the half-live time in water was the smallest for Triazamate, with accompanying high biodegradation rates in soil and sediment. However, the uncertainty was large. This is also reflected in a relative contribution to variance of the MPE of >82 percent for $k_{biodeg,water}$. As a consequence, the persistency, the aquatic PEC, and the MPE have the largest 90%-CI for Triazamate.

The predicted PNEC was based on three multiple linear regressions, i.e. for the LC50 of *Onchorynchus Mykiss*, for the EC50 of *Daphnia Magna*, and for the EC50 of *Pseudokirchneriella Subcapitata*. Typically, *P. Subcapitata* was the most sensitive species for three out of the five triazoles, and *O. Mykiss* for the other two triazoles. However, the uncertainty distribution of the PNEC is not the equivalent of the uncertainty distribution of the most sensitive species. For the most sensitive species, the contribution to variance in the PNEC ranged from 68.5 percent (Bromuconazole in *O. Mykiss*) to 79.7 (Metconazole in *P. Subcapitata*). Furthermore, in four out of five triazoles, the EC50 of *D. Magna* also had a minor influence on the variance in the PNEC and MPE for agricultural soil. These findings emphasize the importance of including species of different trophic levels, rather than choosing one sensitive species.

Appendix 2

Case study 2: Non-testing versus testing based risk assessment on three PBDEs

Introduction

Replacing testing information with non-testing information to support decision making must be done with care. Requirement of strong reliability in risk assessment depend is the consequences of the decision made. For example, there is a difference when QSAR[1] predictions are used to design experiments or to generate hypothesis about possible mechanics, compared to when QSAR predictions are used as weight-of-evidence replacing experimental testing information in risk assessment or waiving. REACH complies with the 3R philosophy to replace, refine and reduce experimental testing on animals. The aim of three R's can be enhanced by studies aimed to address the reliability in replacing testing with non-testing information in risk assessment. Replacing testing with non-testing information may introduce an error in assessed risk, which in turn may lead to less safe, or unnecessary strict, regulatory decisions (the former worse than the latter). Bad decisions may be avoided by considering the uncertainty in non-testing information. That is why an aim with the project CADASTER has been to characterize the uncertainty in QSAR predictions, with the purpose to address the question is considering uncertainty may increase the reliability in non-testing information in risk assessment.

The objective of this case-study is to demonstrate the application of QSARs and QSPRs in probabilistic risk assessment, and the evaluation of reliability in using such non-testing information instead of testing information in regulatory decisions. The use of non-testing information is limited to methods for which uncertainty have been quantified. That is why uncertainty analysis was restricted to chemical specific parameters for which the majority had been predicted QSPRs and QSARs. Uncertainty in assessments for decision making is formed by subjective beliefs relating to the background knowledge, and it means that uncertainty is not quantities that exist independent of the method for measurement. Instead, uncertainty is given a treatment, which means to identify, quantify and respond to when making decisions.

A scientific approach to evaluate the reliability in non-testing information requires an established principle for prediction and thereby a sound basis for quantifying uncertainty in QSAR predictions.

[1] QSAR is often used as a general term including QSPR

Predicting is hard and in this case-study we assume some ideal conditions such as that the conditions for predictive inference are fulfilled for every model. Reliability in non-testing information is assessed in retrospect by studying the consequences in real applications where both kinds of information are present.

Here we demonstrate this on probabilistic risk assessment using the framework for QSAR/QSPR based probabilistic risk assessment developed in CADASTER on three Polybrominated diphenyl ethers (PBDEs), which are one of the CADASASTER classes. The three selected PBDEs were BDE-03(?), BDE-28(2,4,4'-TriBDE) and BDE-47(2,2',4,4'-TetraBDE) and available experimental data were sought for as many of the QSAR /QSPR predicted input parameters as possible. PBDEs belong to an emerging class of organic pollutants widely used, especially in the past, as flame retardants in a variety of consumer products. PBDEs potentially include 209 congeners divided into 10 congeneric groups (mono- to decabromodiphenyl ethers).

Exposure assessment

Environmental fate of the three PBDEs was calculated using the multimedia fate model SimpleBox (H.A. Den Hollander 2004) for a unit emission to air at the regional scale. Non-testing information was provided by QSPRs of chemical-specific properties at 25°C, developed or specified in Work package 3 in CADASTER. QSPR predictions was used to specify Simplebox input parameters water solubility (S, mg/L) (Papa, Kovarich et al. 2009), melting point (Tm, °C) (Papa, Kovarich et al. 2009), vapor pressure (Vp, Pa) (Papa, Kovarich et al. 2009), organic carbon - water partition coefficient (Koc, L/kg) (Gramatica, Giani et al. 2007) and hydroxyl radical reaction rate (k_{OH} , cm3/s.molecule) (Roy, Kovarich et al. 2011). The QSPRs for K_{oc} and k_{OH} are given in Table TRIA_1, whereas QSPRs for Tm, S and Vp are reported in the Table 1.

Table 1. Selection of QSPRs for physico-chemical properties of PBDEs at 25°C (Papa, Kovarich et al. 2009).

Parameters	units	R^2 %	Model description
Melting point (T_m)	°C	84.37	T_m = 1968.06 – 6227.09 X2A
Water solubility (S)	mol/L	91.80	log 1/S = 6.09 – 1.18 Mor23m
Vapor pressure (V_p)	Pa	98.71	log 1/V_p = 0.115 + 0.213 T(O…Br)

X2A = average connectivity index chi-2

Mor23m = Morse signal no 23 weighted by atomic masses

T(O…Br) = sum of topological distances between oxygen and bromine atoms

Biodegradation in water was predicted by the ultimate biodegradation estimation model, BIOWIN3, included in the estimation software EPI Suite™ (Boethling, Howard et al. 2004). The BIOWIN3 classify a compound into a biodegradation category based on molecular fragments. However, Simplebox uses biodegradation half-live in surface waters (τ_{wat}, days) as input parameter and not a category. Therefore, values for half-live was assigned based experimental data on half-lives collected and described for each of the eight BIOWIN3 categories (Aronson, Boethling et al. 2006). Half-lives in sediment and soils were assessed from the half-lives in water by assuming it to be two times higher in soils and nine times higher in sediments, respectively as it is commonly done in EPI Suite™. Concerning the applicability domain of BIOWIN in EPI Suite, there is no well-defined criterion about the reliability of the predictions. It is only mentioned there about the model domain that the user may wish to consider the possibility that biodegradability estimates are less accurate for the compounds outside the molecular weight (MW) range of the training set compounds.

Models to assess fate of PBDEs have for long only considered the OH reaction rate constants in the gas phase (Gouin and Harner 2003; Wania and Dugani 2003), but the photolysis in the atmosphere have been suggested to be a critical parameter in the assessment of PBDEs (Eriksson, Green et al. 2004; Raff and Hites 2007; Schenker, Soltermann et al. 2008). Photolysis of PBDEs in air has been seen in laboratory experiments but since field studies are difficult, there are no experimental data available that correspond to field conditions. Fate assessments on PBDEs have for long been done without considering photolysis (Palm, Cousins et al. 2002; Gouin and Harner 2003; Wania and Dugani 2003), but recently an assessment considering photolysis showed photolysis may be important (Schenker, Soltermann et al. 2008). A QSAR for the photolytic half-life in air ($\tau_{photo,}$ 1/s) were given by a linear regression on the adsorption spectra and quantum yield measurements in the atmosphere of two compounds Di-BDE3 and Tri-BDE-7, resulting in a negative slope with increasing homologues of PBDEs (Raff and Hites 2007). This regression is based on a simplification and have been applied in risk assessment where 209 PBDE congeners were grouped in homologues that made it easier to handle the photo-degradation in the fate assessment (Schenker, Soltermann et al. 2008). The predictions of the regression model were observed within the estimates of photolytic rates for PBDEs.

Reliability in QSPR predictions

The models in Table 1 also fulfil the OECD principles (OECD. 2006). The third OECD principle states that a model should have a well-defined domain of applicability (AD). Here we ask what it means when applying the model in risk assessment. Following the practice suggested by several authors

(Eriksson, Jaworska et al. 2003; Papa, Kovarich et al. 2009), reliability in using these five QSPR models to predict the three compounds under consideration were judged by the leverage approach (Table 2). This means that leverage is calculated from model descriptors for the training data set and the selected BDE in question as the sum of the diagonal of the hat matrix. A leverage value can geometrically be seen as a distance in space spanned by the descriptors, and is a measure of the extent of extrapolation.

In order to judge whether an item is close enough, it has been suggested to compare leverage value to a cut off c·p/n where p is the number of model descriptors (including the intercept), n is the number of points in the training data set, and c is a constant. In Table 2 the cut off was defined by setting c = 3, as suggested by Gramatica and others. A well-established software for chemo informatics uses c = 3 as a default, but say that any value between 1 and 10 are possible (Martens and Næs 1989). However, a clear cut value on c may cause problems in practical applications, especially since compounds to predict quite often are distant to the model, and in a sense close to being extrapolated.

Based on c = 3, the three PBDEs fell inside the AD for the models predicting S, Tm, Vp and Koc. The QSPR of K_{OH} had been trained on Volatile organic compounds (Gramatica, Pilutti et al. 2004)., and it has been shown that most PBDEs are not found inside the AD (Roy et al , 2011). However, the predictions showed a good agreement with the predictions obtained from EPI Suite verified by the Insubria group showing the difference in the predictions for PBDEs was within 0.8 log unit. The predictions from EPI Suite and the QSPR model for k_{OH} were similar but the crucial information on AD for chemicals, obtained from the Insubria model, was an advantageous aspect. Given that the PBDEs were outside the AD for k_{OH}, the use of these predictions also needs to be evaluated in light of the sensitivity of the assessed risk to the input parameter k_{OH}.

Table 2. Assessment of Applicability Domain of PBDEs using Leverage approach. Bold values means that a compound is outside the AD for a value on c = 3.

Parameters	S	Tm	Vp	K_{oc}	k_{OH}
Cut off	0.500	0.240	0.180	0.023	0.032
BDE-03	0.229	0.181	0.110	0.003	**0.040**
BDE- 28	0.191	0.108	0.031	0.006	**0.045**
BDE- 47	0.115	0.071	0.031	0.007	**0.057**

Uncertainty in QSPR predictions

A statistical and quantitative approach to assess the error in a prediction involves predictive inference. The Bayesian framework for predictive inference is despite the problems of predicting in general (see Appendix 1) pointed out as the most robust and reliable framework that quantify the uncertainty in a prediction by a probability distribution. Bayesian inference provides an output with an interpretation that is in agreement with the interpretation of risk assessor, but most importantly decision makers. However, Bayesian inference is not the dominating statistical principle in QSAR modelling, and it does not have to be. Bayesian inference is useful when applying QSARs in decision making, such as to support uncertainty analysis in chemical safety assessment. The step from a documented QSAR model to an applied situation using predictive inference has been identified in WP4 CADASTER as crucial to integrate QSARs in risk assessment and was addressed in the CADASTER deliverable D4.2. It can be shown that when uncertainty in a QSPR prediction from a regression fitted by OLS is assigned a t-distribution defined by the predicted point estimate and predictive error (see Eq 6), well approximates corresponding predictive distribution from Bayesian inference under certain conditions. To circumvent the problem of "Bayesianise" the QSAR models, predictive means and errors for the physic-chemical properties and corresponding degrees of freedom, were here used to define the uncertainty in those input parameters predicted by OLS (Table 3).

Table 3. Quantification of uncertainty in physico-chemical properties, atmospheric degradation rates and biodegradation half-lives at 25°C for the selected PBDEs.

PBDE	T_m [a] (°C)		log S [a] (mol/L)		log V_p [a] (Pa)		log K_{oc} [b] (L/kg)		log k_{OH} [c] ($cm^3 s^{-1}$ per Molecule)		Biodegradation half-lives in water [b] ($\tau_{w,}$ days)		Photolytic degradation rate[c] (k_{photo},1/s)
n / p [d]	25/1		12/ 1		34/1		643/4		460/4		Qualitative model	Lognormal distribution Based on experimental data	2/1
	PRED	SEP	PRED	SEP	PRED	SEP	PRED	SEP	PRED	SEP	BIOWIN[3] Category[f]	(M, CV)	
BDE-03	43.88	21.44	–6.73	0.27	–1.18	0.17	3.55	0.56	–11.42	0.44	Weeks-months	(20, 7.45)	1.34E-05
BDE-28	68.79	20.77	–6.97	0.26	–2.88	0.16	4.10	0.56	–11.97	0.44	Months	(85,1.96)	1.34E-05
BDE-47	87.47	20.42	–7.35	0.25	–3.52	0.16	4.34	0.56	–12.26	0.45	Recalcitrant	(88,1.91)	3.38E-05

a Papa et al. (2009), b Gramatica et al. (2007), c Roy et al. (2011), d Aronson et al. (2006), e Raff and Hites (2007).

n = number of compounds in training data, p = number of descriptors for which degrees of freedom in a t-distribution is n-p-1

f: Division of recalcitrant category with respect to BIOWIN output ; M = median; CV = coefficient of variance; PRED: predictive mean, SEP: predictive error

Default values were given to the all parameters in the Simplebox model relevant for exposure in aquatic compartment, except for half-life for biodegradation and photolytic rate. These two input parameters were specified by expert judgment supported by QSPR predictions and experimental data. The uncertainty in half-life for biodegradation was assigned by a lognormal distribution based on analysis of experimental data by Aronson et al. (Aronson, Boethling et al. 2006) that were used to revise the categories of biodegradability in the predictions of biodegradation BIOWIN3 (in EPISUITETM) (Table 3). A correlation of 1.0 was assumed among the half-lives in water, soil and sediment in order to quantify the uncertainty in soil and sediment by multiplying with factor 2 and 9 respectively.

Photolysis was assigned a single value, which was regarded as an upper bound. This was motivated by seeing that the prediction by Raff and Hites was based on a model fitted to upper limits of photolysis estimates and that half-life decrease with the number of bromine atoms increase. No half-life of PBDE was used as a conservative lower bound. In this way the uncertainty in half-lives were quantified as an interval stating that the actual value can fall anywhere inside the interval with no specifications of one value being more likely than another. In practice two different risk assessments were done, one with photolysis at a large value and one without photolysis. Predicted Environmental Concentration (PEC) was obtained as a pair of cumulative probability distributions (CDFs), and it was verified by further simulations that these CDFs form a probability box (p-box) bounding all possible probability distributions generated by values on photolysis within this interval.

Sensitivity analysis of exposure

Sensitivity analysis was performed with the purpose to compare non-testing based risk assessment with experimental based risk assessment. This sensitivity analysis was performed on the two scenarios for photolysis which was the upper and lower bounds of a p-box. Input parameters for which such information was available were described by predictive distributions from both QSPR and QSAR, and experimental data from direct tests on the compound in question. There were physico-chemical experimental data available for BDE-28 (Tm 64.25; log S -6.76; log Vp -2.8) and BDE-47 (Tm 82.58; log S -7.51; log Vp -3.5) since these two compounds had been present in the training or validation data set used to develop some of the QSPR models. Since experimental data available on melting point, solubility and vapor pressure were well covered by the corresponding predictive distributions, the reason to why uncertainty distributions based on QSPR predictions were wider in comparison to testing based distributions, was that uncertainty was not considered in testing information. Uncertainty in experimental data may be large. Uncertainty in experimental data has been raised as an important aspect in QSAR modeling that potentially may improve models predictivity and reliability.

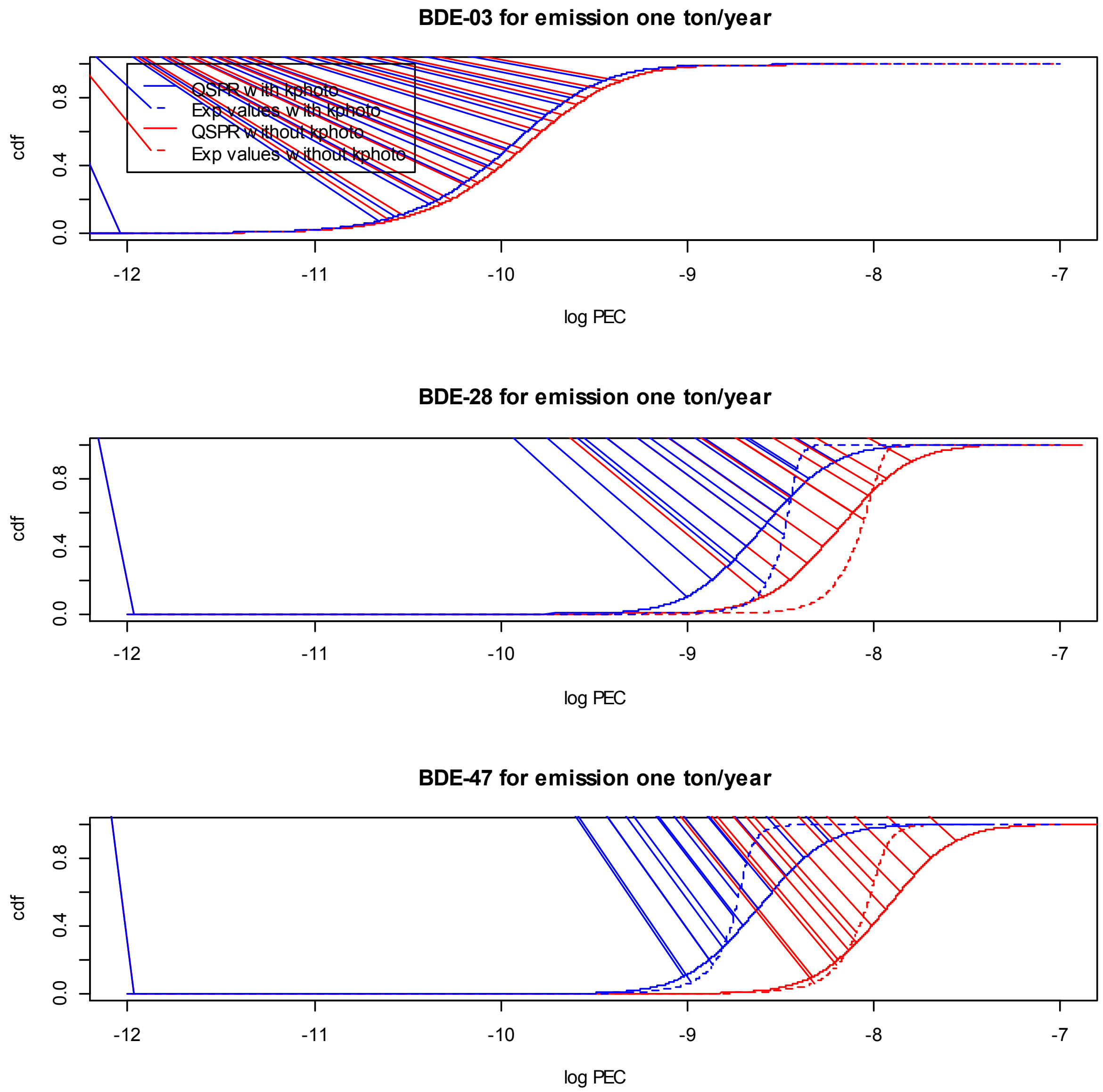


Figure 1. Comparison of log PEC (mg/l) for non-testing (QSPR predictions) and available testing information (experimental data instead of QSPR predictions when possible) with and without photolysis in fresh water (based on a unit emission in ton/year).

Effect assessment

A model to handle uncertainty in input data for SSD is under development. Meanwhile we fitted a SSD to point predictions. For BDEs there were 3 QSAR models available to predict acute and chronic effects available from ECOSAR (Table QSAR_BDE in Appendix). None of the three BDEs were included in the training data set for ECOSAR, and testing information to assess the effect in aquatic environment was searched for in other sources. Experimental acute values were found for BDE-03 on the species Daphnid and Fish, BDE-28 on Nitocra spinipes, and BDE-47 on fundulus heterocliticus

(Table QSAR_BDE). PNEC values were calculated by adding uncertainty factors when needed (Table QSAR_BDE). A SSD for non-testing and testing information was derived by adjusting the mean of the fitted SSD on predicted chronic effects on three species according to the ratio between PNEC values derived based on the predicted acute and chronic and between predictions and experimental acute (Appendix 4). This somewhat ad hoc procedure was regarded as acceptable for this case-study were the purpose was to the demonstrate how to evaluate the sensitivity on risk to testing and non-testing information.

Sensitivity analysis on effect

For BDE-03 two experimental values on Fish and Daphnids were available, and there were only a small difference between non-testing and testing based effect (Figure 2). For BDE-28 non-testing information lead to a relatively higher hazard potential of the chemical, but the difference was small. Finally, there was a large difference in hazard potential based on non-testing and testing information for BDE-47, which comes from the adding of uncertainty factors to experimental estimates were derived based on a study of a single species that had been limited by water solubility, and therefore the PNEC had been given to be at least greater than the value (see Table SSD.BDE in appendix). Thus, the changes in SSDs based on non-testing compared to testing information are quite different for the three BDEs.

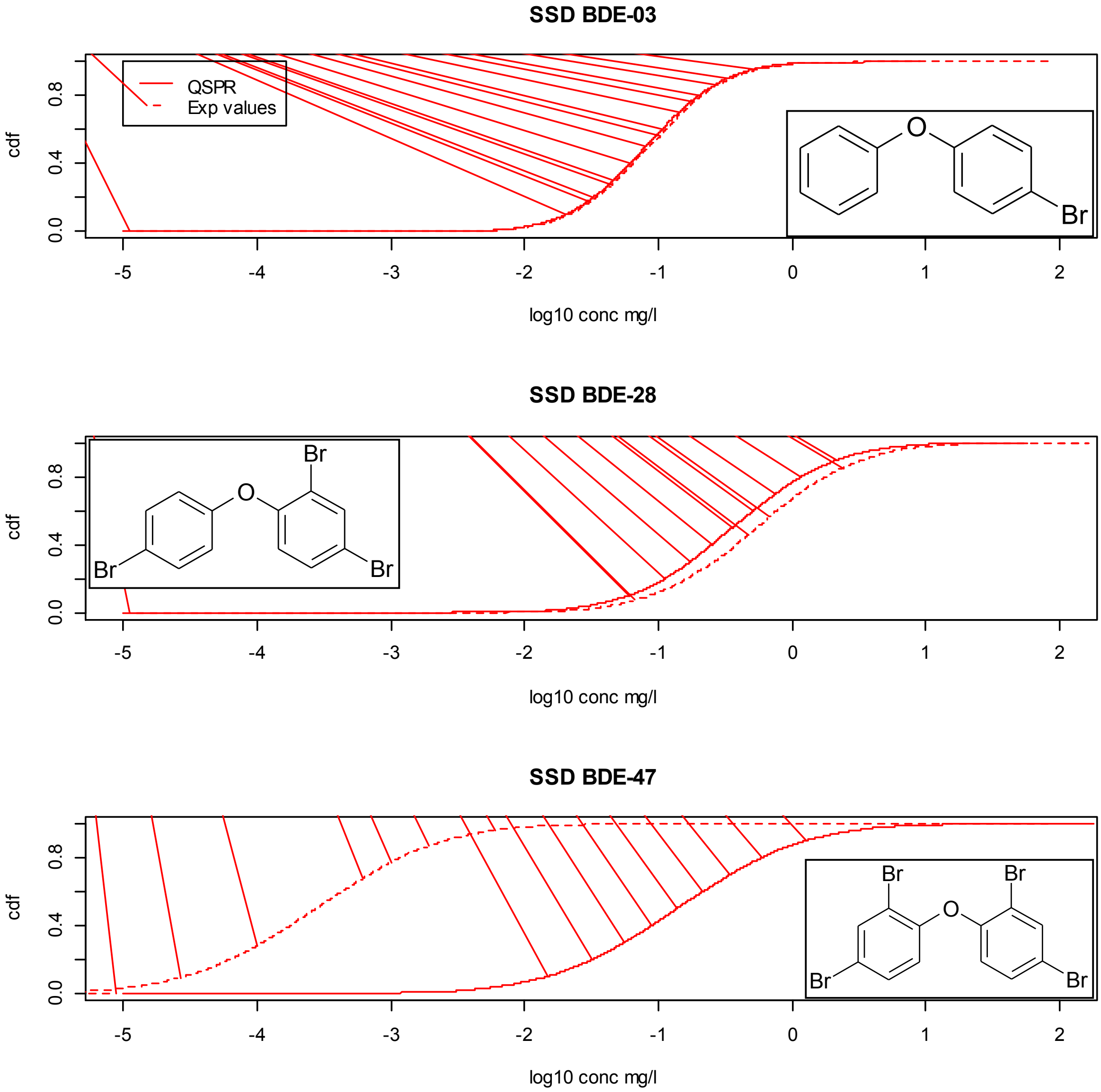


Figure 2. Species Sensitivity Distribution based on QSAR predictions (solid) and experimental values (dashed).

Risk assessment

Environmental risks are typically estimated in a deterministic way using single point estimates for both exposure (PEC) and effects (PNEC). For uncertainty analysis these point estimates (PEC and PNEC) are replaced by probability distributions. Uncertainty in PEC is derived by Monte Carlo simulation of the Simplebox model based on the specified uncertainty in input parameters. PNEC are recommended to be derived as a Species Sensitivity Distribution (SSD) in order to capture the variability between species in the ecological system. The probabilistic PEC and PNEC (here derived for an aquatic environment) are now combined into a quantitative risk measure. The deterministic measure Risk Characterization Ratio (RCR) is hampered by not being a measure of risk - it is sensitive to scaling and is therefore not comparable between substances. A probabilistic measure of risk is the probability of an undesired effect P(PEC > PNEC). This probability can alternatively be expressed as the Expected Risk (Figure 3), which is the expected fraction of species affected for an uncertain exposure (Appendix TOM).

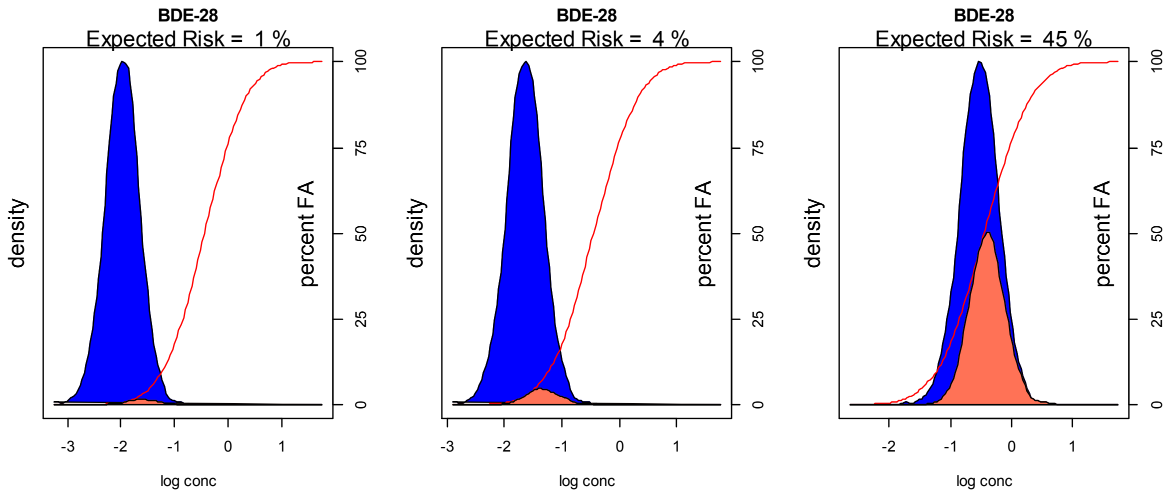


Figure 3. Expected risk is a measure to what extent the PEC and PNEC distributions overlap, has a clear interpretation in terms of the expected fraction of species affected, and is invariant to scale which facilitates comparison between different risk assessments.

Sensitivity analysis on Expected Risk

Risk depends on the rate of emission of the chemical into different compartments. Expected Risk was calculated on a range of alternative emission scenarios in air on a regional scale. Assuming that emission scenarios resulting in a risk less than 5% are regarded as safe, non-testing-based ER were compared to testing-based ER by searching for discrepancies in the following regulatory decision for different emission scenarios. Here the results based on PEC assessed without considering photolytic rate into air was used. As seen in Figure 4 there are emission rates for which there is a discrepancy between non-testing and testing based probabilistic risk assessments. For example, an emission of 5 ton BDE-28 into air per year would be regarded as safe if based on testing information, while regulatory actions would be necessary if the decision had been based on QSAR and QSPR predictions. Correspondingly, the same emission of BDE-47 would be regulated when based on testing information, while regarded as safe when based on non-testing information. This is an example of when limited information from experimental testing result in an conservative risk estimate, which would have been less conservative by applying a weight-of-evidence approach and allow the PNEC be assessed based on additional consideration of a QSAR-based PNEC. A discrepancy between testing and non-testing based risk for likely emission rates is an indication that further testing is needed.

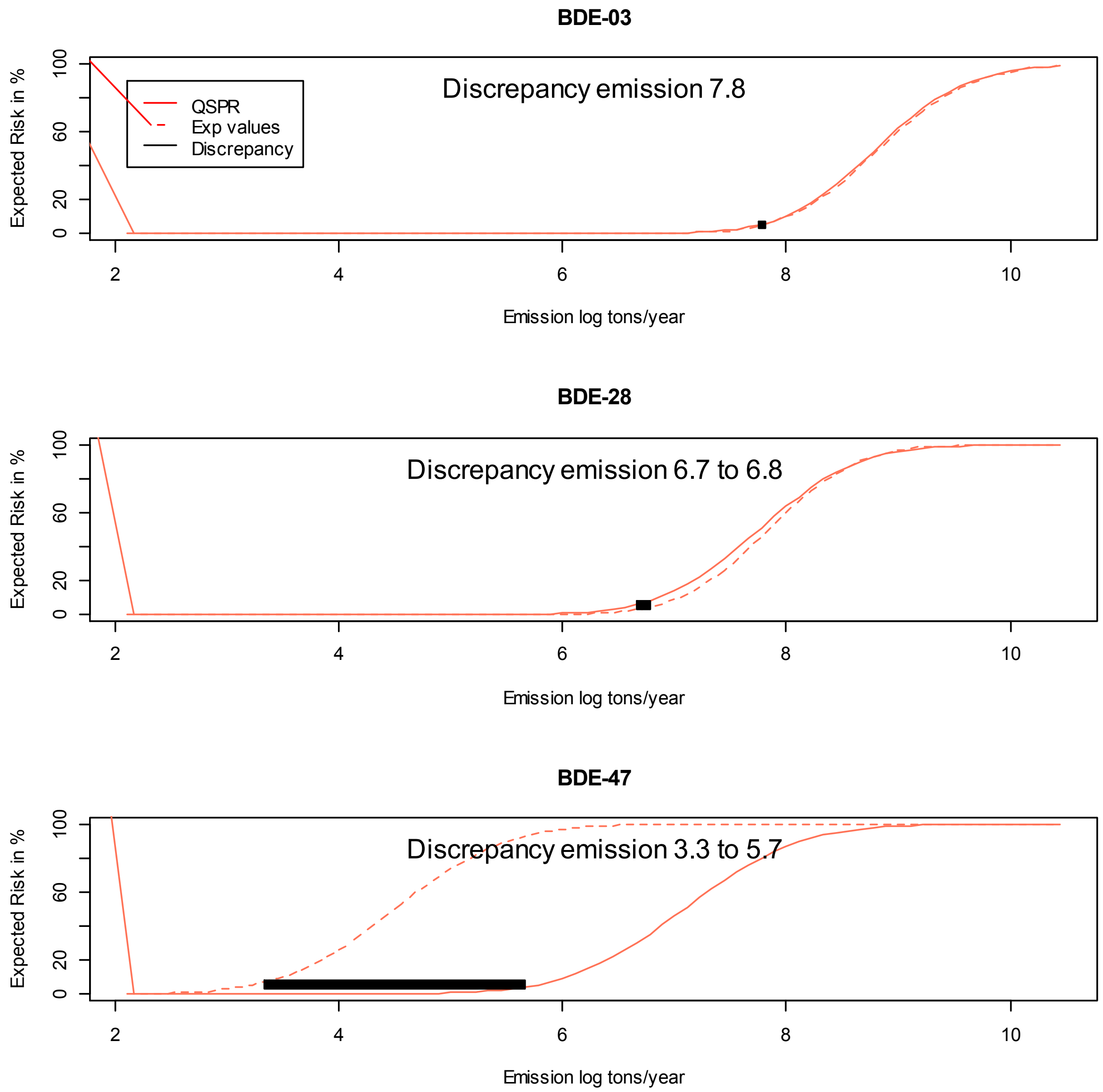


Figure 4. Discrepancy in regulatory decision when Expected Risk is derived from testing versus non-testing information for three BDEs.

Conclusions

This case-study exemplifies the regulatory consequences of using non-testing information in the absence of testing information, but can also be seen as the consequences of combining non-testing information with weak testing information as a weight-of-evidence approach. A small discrepancy between non-testing and testing based risk assessment may not only be an effect of accurate QSAR predictions. For example, when the influence of a single parameter is small in comparison to other parameters in the assessment, whether testing or non-testing information is used does not make a large difference on the risk. Identifying which parameters with a large influence on risk or its uncertainty can be approached by different kind of sensitivity analysis, such as the one in the case-study of Triazoles. QSAR uncertainty needs to be put in perspective to other uncertainties. The exposure assessment of the three BDEs show that the influence of QSPR predicted parameters are small in comparison to whether or not photolytic rate in air should be considered. In order to generalize the impact of non-testing information provided by QSARs in chemical risk assessment the approach described here will be done on a larger set of chemicals carefully selected to represent chemical space by experimental design (Appendix 4). General conclusions on the reliability of non-testing information in risk assessment will be difficult to make, since the importance of different sources of information depends on each other, the context for the assessment and the decisions made.

Appendix 3

Case study 3: Prioritization based on PBT evaluation on BDEs and uncertainty analysis

Introduction - Relative PBT scores

Ranking BDEs according to urgency of concern and urgency of further testing in order to increase the confidence in the ranking, was performed based on relative PBT scores derived from QSAR evaluated endpoints used as in the PTB classification under REACH (Table 1). Relative PBT scores were assigned by relative scores based on the rank of the compounds according to the P, B and T in an increasing order. A relative PBT score was assigned to be the product of these scores, i.e.

PBT score = rank {in ascending order} (P score * B score * T score).

Evaluation of confidence in QSAR predictions

A QSAR predicts compounds with more or less confidence dependent on to what extend a compound is an extrapolation from the model's domain of applicability. Predictive Reliability (PR) in individual QSAR predictions can be evaluated based on measures of extrapolation such as distance to the model. When QSARs are used for input parameters in an assessment, we evaluated the total predictive reliability by weighting the relative importance of each QSAR prediction to the output from the assessment. Low predictive reliability was propagated through an assessment model by enlarging the predictive distribution, keeping the median fixed. The change in the assessment output by enlarging the error in unreliable predictions was used as a score of predictive reliability. Assessments based on QSARs where the compound fall inside the applicability domain of all QSARs were assigned a zero score value. The existence of low predictive reliability was then evaluated in a similar way as the PBT score, i.e. as a ranked sum

PR low = rank {in ascending order} (PR score P + PR score B + PR score T)

Persistence assessment

Overall persistence was assessed by the fate model Simplebox, commonly used within REACH (Iqbal and Sahlin 2012). QSARs were used to predict the physicochemical and toxicological properties of the PBDEs. The predictions were provided by the available QSARs for the physicochemical properties namely water solubility (S, mg/L) (Papa et al., 2009), vapor pressure (Vp, Pa) (Papa et al., 2009), organic carbon-water partition coefficient (Koc , L/kg) (Gramatica et al., 2007), and hydroxyl radical reaction rate (k_{OH} , cm^3*s^{-1}/molecule) (Roy et al. 2011), measured at 25°C in addition to biodegradation in water (τw , days) (See Table 1). The hydroxyl radical concentration was taken 106 molecules per cm3. It is worth noticing that QSARs for S and Vp were specifically developed for PBDEs and QSARs for k_{OH} and Koc were applied to PBDEs. Apart from that these model fulfill the OECD principles for validations of QSARs making them more appropriate to use them in the assessment. For degradation in aquatic environment, the geometric mean using the linear regressions for BIOWIN, a part of EPISUITETM, outputs derived by Arnot et al. (2005) was taken into account. Half-lives in sediment and soils were estimated on the basis of the half-lives in water, assuming it to be two times higher in soils and nine times higher in sediment. These are the multiplying factors used by the US EPA profiler.

Table 1. Description of the PBT classification.

Classification endpoint	Ranking quantity	Assessments
Persistence	Pov (days)	Assessment: Pov from level III model, QSARs: several see text, Predictive distribution: Statistical and expert assigned, Predictive reliability: Evaluated for each QSAR by distance to model and propagated through the assessment by lower the confidence for unreliable predictions, evaluated by sensitivity analysis
Bio-concentration	Bio-concentration factor log BCF	QSAR (Mansouri, Consonni et al. 2012), Predictive distribution not assessed, Predictive reliability provided.
Toxicity	-HC (mg/L)	Assessment: HC from SSD approach, QSARs: ECOSAR Fish, Daphnia and Algae, Predictive distribution: Student-t associated to OLS regression, Predictive reliability: Distance to model determined by hat values and other criteria defined by ECOSAR

*a negative sign was added to have all the ranking quantities to increase with hazardous potential

Bioconcentration assessment

Bioconcentration factor was assessed as point predictions from a QSAR developed for BDEs (Mansouri et al., 2012). Predictive reliability had been evaluated by distance to the model.

Toxicity assessment

The aquatic toxicity of the chemicals for the chronic effects is estimated from ECOSAR. The chronic effects of Fish, Daphnid, and Algae were considered in the ECOSAR. The regenerated QSARs for each species using the experimental data in the background of ECOSAR used to consider the error in the data. Hazardous concentration was derived as the 5th percentile (HC05) of a log normal Species Sensitivity Distribution fitted to three species.

Predictive reliability in toxicity assessment was evaluated by enlarging the uncertainty in individual toxicity values when something fell out of the applicability domain of a QSAR, and calculating the relative influence on the final toxicity score.

PBT scores and ranking

Different congeners of BDEs are identified by the number of bromine atoms. There is a positive relationship between number of bromine atoms and molecular weight and octanol-water partitioning coefficient (Figure 1), both are used as descriptors in the underlying QSARs or in the assessments.

The ranking suggests that out of these compounds is BDE-153 of most concern (Figure 2). Risk managers seek to avoid missing to give high concern to a highly hazardous compound, i.e. to commit errors of type II. Here it would mean to be careful about compounds assigned a low relative PBT and a low predictive reliability, which means that the relative PBT of this compound may be underestimated. Thus a highly uncertain rank, like BDE-203 could be given higher priority. A list of rankings is found in Table 2.

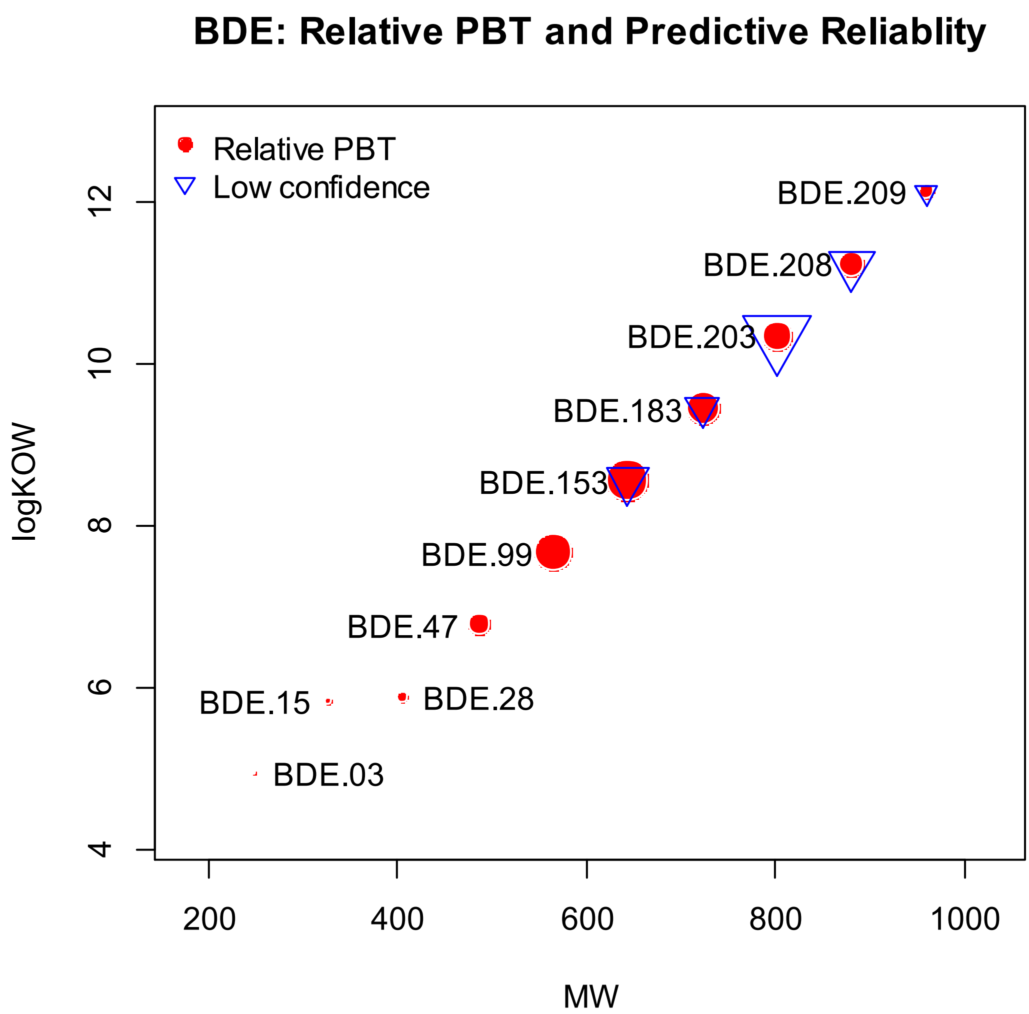


Figure 1. Relative PBT scores including relative confidence in assessments seen over molecular weight and octanol-water partition coefficient.

Table 2. Prioritization among 10 selected BDEs based on relative PBT evaluation.

Congener	Rank Bioaccumulation	Rank Persistence	Rank Toxicity	Total Rank
BDE.03	4	1	1	1
BDE.15	7	2	2.5	2
BDE.28	9	3	2.5	4
BDE.47	8	4	4	5
BDE.99	6	5	5	7
BDE.153	10	6	6	10
BDE.183	5	8	7	8
BDE.203	2	9	8	6
BDE.208	1	7	9	3
BDE.209	3	10	10	9

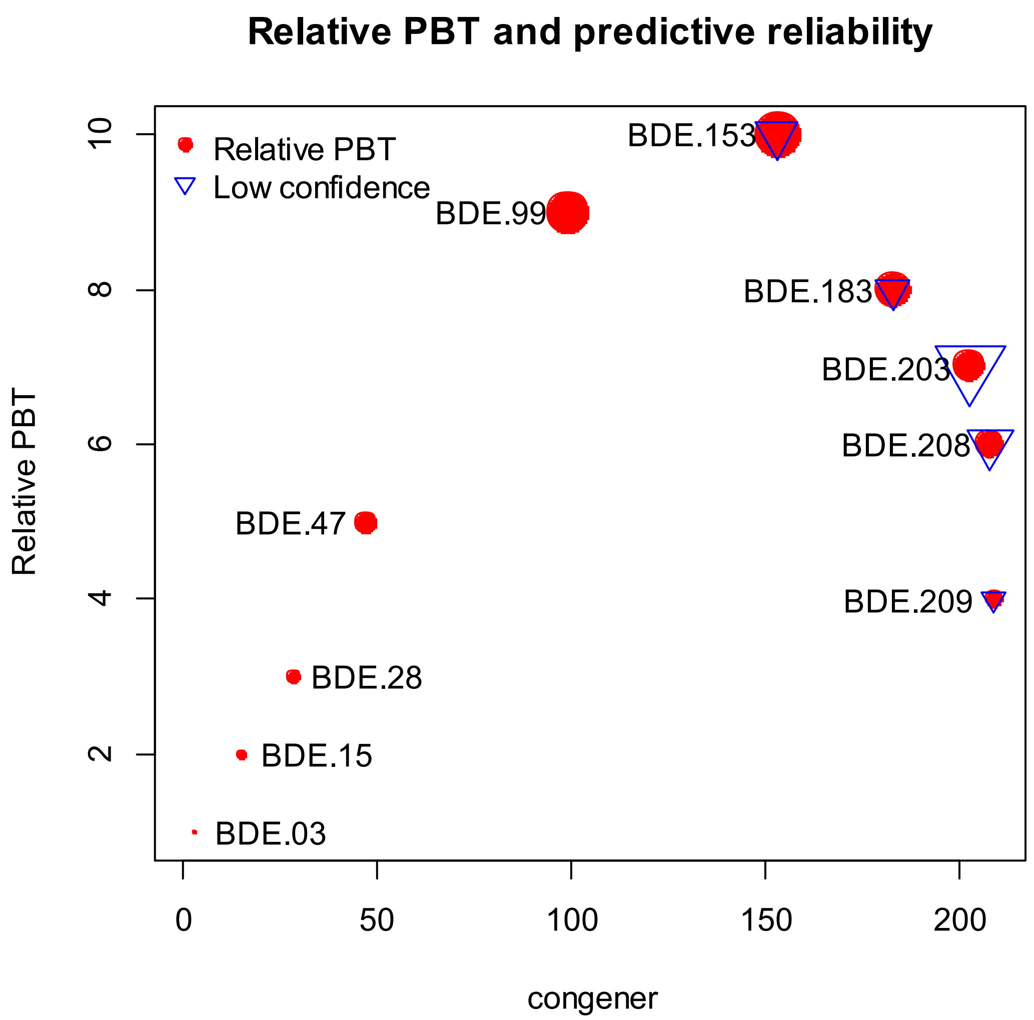


Figure 2. Relative PBT scores including relative confidence in assessments seen over an increasing trend in number of bromine atoms (a main feature between difference congeners).

Appendix 4

Case study 4: Prioritization based on hazard assessment of BTAZs

Overview

Hazards were assessed for the 386 compounds constituting the CADASTER list of (benzo)triazoles. The assessments was based on PNEC values, calculated from QSAR predictions of fish, algae and daphnia. QSAR predictions had been generated from a consensus QSAR developed within the CADASTER project (Cassini, Kovarich et al. in review), and available via the CADASTER website. PNEC-values for each of the compounds were assessed as the minimum EC_{50} out of the three species with an uncertainty factor of 1000, according to common procedure in hazard assessment.

Confidence in every assessment was evaluated by the relative frequency of models for which a compound fell out of the corresponding applicability domain, summed over the species.

AD.index = Sum(out of AD) / total number of QSARs in a consensus prediction for fish + Sum(out of AD) / total number of QSARs in a consensus prediction for algae + Sum(out of AD) / total number of QSARs in a consensus prediction for daphnia

This index was used to identify compounds whose assessments for which confidence is low.

The main finding of the study is that relative Hazard increased with molecular weight (Figure 1). The confidence in assessments was lower for the most extreme hazards (Figure 2). The final ranking of a list of 386 BTAZs is given in Table 1.

References

Cassani, S., S. Kovarich, et al. (in review). "Evaluation of CADASTER QSAR models for aquatic toxicity of (benzo-)triazoles and prioritization by consensus."

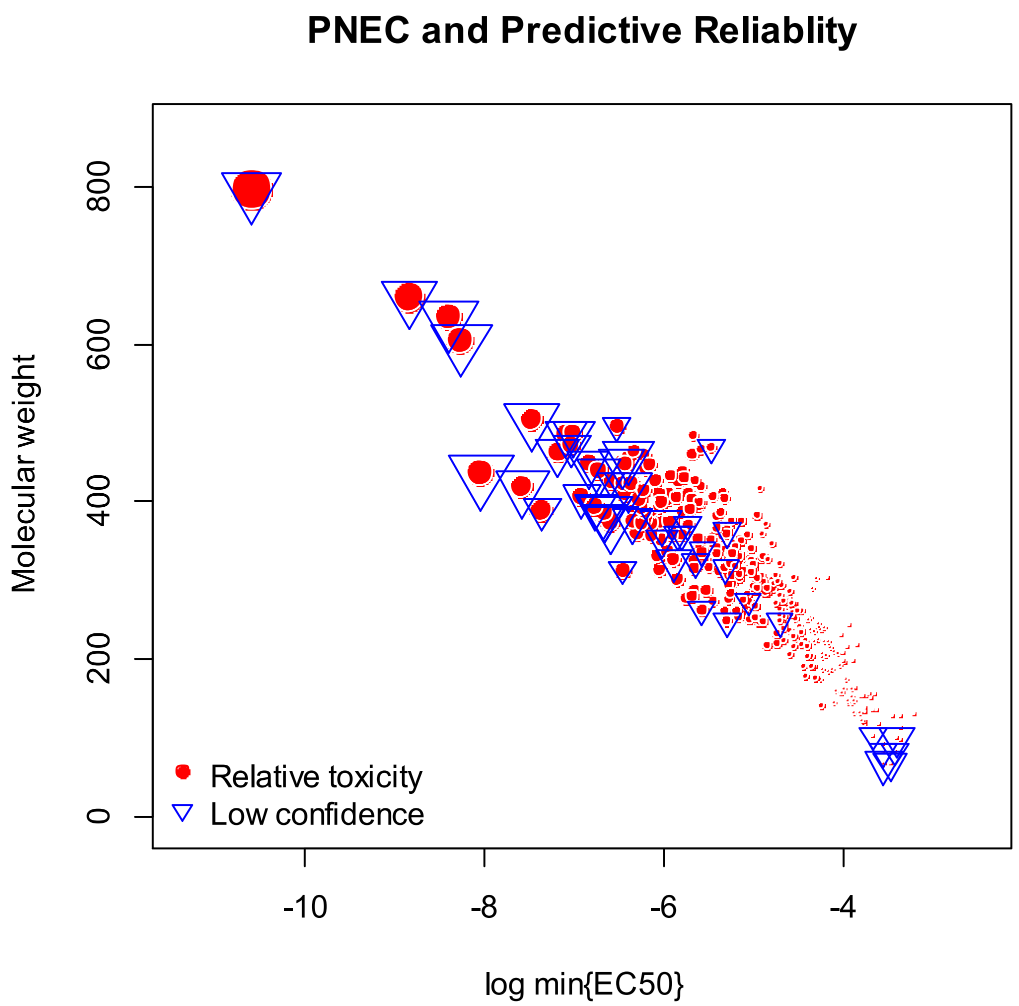


Figure 1. The distribution of relative hazards and relative confidences in predictions over the molecular domain and hazard endpoint.

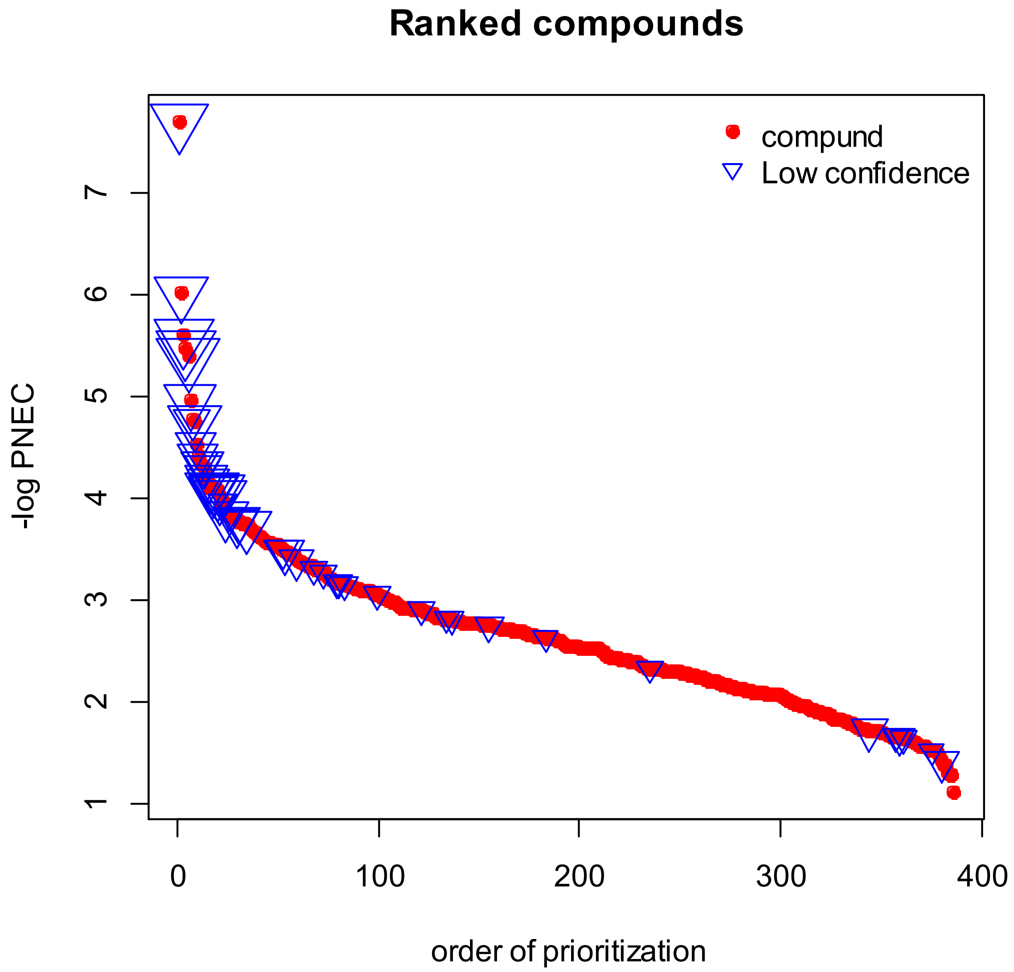


Figure 2. Ranking of compounds based on relative hazard indicating compounds with lower confidence in their assessments.

Table 1. BTAZ prioritization based on relative hazard.

ID	CAS	min(EC50) mol/L	min(EC50)/1000 mol/L	MW (g/mol)	PNEC (mg/L)	log PNEC (mg/L)	low predictive reliability	Relative Hazard (-log PNEC)	Rank
22	001325-58-2	-10,60	2,51E-14	796	2,0E-08	-7,70	2,43	7,70	1
302	103597-45-1	-8,84	1,45E-12	659	9,6E-07	-6,02	2,27	6,02	2
5	000131-43-1	-8,40	4,01E-12	633	2,5E-06	-5,60	2,47	5,60	3
177	063216-86-4	-8,26	5,54E-12	603	3,3E-06	-5,48	2,47	5,48	4
123	041083-11-8	-8,04	9,17E-12	436	4,0E-06	-5,40	2,60	5,40	5
18	000974-29-8	-7,58	2,64E-11	418	1,1E-05	-4,96	2,20	4,96	6
38	003333-62-8	-7,36	4,39E-11	389	1,7E-05	-4,77	1,50	4,77	7
69	006994-51-0	-7,47	3,42E-11	504	1,7E-05	-4,76	2,27	4,76	8
143	051627-14-6	-7,19	6,41E-11	463	3,0E-05	-4,53	1,73	4,53	9
236	083044-89-7	-7,10	7,88E-11	486	3,8E-05	-4,42	1,73	4,42	10

239	083366-66-9	-7,03	9,43E-11	470	4,4E-05	-4,35	1,53	4,35	11
237	083044-90-0	-7,01	9,67E-11	486	4,7E-05	-4,33	1,73	4,33	12
62	005516-20-1	-6,92	1,21E-10	407	4,9E-05	-4,31	1,53	4,31	13
193	070321-86-7	-6,85	1,42E-10	448	6,3E-05	-4,20	1,73	4,20	14
317	125304-04-3	-6,77	1,72E-10	394	6,8E-05	-4,17	1,73	4,17	15
33	003142-42-5	-6,67	2,12E-10	380	8,1E-05	-4,09	1,73	4,09	16
319	127519-17-9	-6,73	1,85E-10	438	8,1E-05	-4,09	1,73	4,09	17
226	080595-74-0	-6,68	2,10E-10	387	8,1E-05	-4,09	2,07	4,09	18
225	080584-90-3	-6,67	2,12E-10	387	8,2E-05	-4,09	2,23	4,09	19
249	094270-86-7	-6,67	2,13E-10	387	8,2E-05	-4,08	2,23	4,08	20
222	080301-64-0	-6,60	2,50E-10	373	9,3E-05	-4,03	2,23	4,03	21
140	042509-80-8	-6,47	3,40E-10	314	1,1E-04	-3,97	1,07	3,97	22
85	016515-58-5	-6,60	2,51E-10	426	1,1E-04	-3,97	1,13	3,97	23
4	000130-34-7	-6,48	3,28E-10	450	1,5E-04	-3,83	1,93	3,83	24
351	141079-20-1	-6,53	2,98E-10	497	1,5E-04	-3,83	1,13	3,83	25
339	141079-03-0	-6,46	3,51E-10	424	1,5E-04	-3,83	0,40	3,83	26
334	141078-95-7	-6,44	3,67E-10	410	1,5E-04	-3,82	0,40	3,82	27
346	141079-15-4	-6,44	3,59E-10	449	1,6E-04	-3,79	0,20	3,79	28
320	128625-52-5	-6,36	4,40E-10	376	1,7E-04	-3,78	1,67	3,78	29
178	063251-40-1	-6,40	3,99E-10	420	1,7E-04	-3,78	1,93	3,78	30
341	141079-07-4	-6,39	4,10E-10	424	1,7E-04	-3,76	0,40	3,76	31
147	054028-84-1	-6,31	4,86E-10	361	1,8E-04	-3,76	0,73	3,76	32
309	113518-46-0	-6,34	4,61E-10	388	1,8E-04	-3,75	0,80	3,75	33
187	066975-54-0	-6,40	3,94E-10	457	1,8E-04	-3,74	2,07	3,74	34
89	019683-09-1	-6,31	4,90E-10	379	1,9E-04	-3,73	0,77	3,73	35
340	141079-06-3	-6,30	4,99E-10	404	2,0E-04	-3,70	0,77	3,70	36
335	141078-99-1	-6,25	5,61E-10	370	2,1E-04	-3,68	0,77	3,68	37
349	141079-18-7	-6,33	4,64E-10	463	2,1E-04	-3,67	0,57	3,67	38
337	141079-01-8	-6,26	5,56E-10	390	2,2E-04	-3,66	0,40	3,66	39
332	141078-93-5	-6,20	6,25E-10	376	2,3E-04	-3,63	0,40	3,63	40
336	141079-00-7	-6,21	6,17E-10	386	2,4E-04	-3,62	0,60	3,62	41
344	141079-13-2	-6,23	5,95E-10	414	2,5E-04	-3,61	0,20	3,61	42
330	141078-91-3	-6,14	7,24E-10	355	2,6E-04	-3,59	0,57	3,59	43
108	031251-03-3	-6,15	7,06E-10	379	2,7E-04	-3,57	0,57	3,57	44
348	141079-17-6	-6,23	5,85E-10	458	2,7E-04	-3,57	0,40	3,57	45
331	141078-92-4	-6,14	7,26E-10	371	2,7E-04	-3,57	0,60	3,57	46
347	141079-16-5	-6,20	6,36E-10	442	2,8E-04	-3,55	0,40	3,55	47
232	081518-32-3	-6,07	8,53E-10	330	2,8E-04	-3,55	0,20	3,55	48
95	024017-47-8	-6,05	9,01E-10	313	2,8E-04	-3,55	0,33	3,55	49
350	141079-19-8	-6,17	6,82E-10	446	3,0E-04	-3,52	0,40	3,52	50
231	081518-31-2	-6,02	9,50E-10	328	3,1E-04	-3,51	0,40	3,51	51
97	025973-55-1	-6,03	9,26E-10	352	3,3E-04	-3,49	1,33	3,49	52
213	078218-61-8	-6,04	9,07E-10	373	3,4E-04	-3,47	1,67	3,47	53
358	173980-17-1	-6,10	7,96E-10	427	3,4E-04	-3,47	0,20	3,47	54
343	141079-12-1	-6,06	8,80E-10	394	3,5E-04	-3,46	0,40	3,46	55

342	141079-08-5	-6,06	8,79E-10	408	3,6E-04	-3,45	0,40	3,45	56
298	099793-38-1	-5,97	1,08E-09	336	3,6E-04	-3,44	0,00	3,44	57
345	141079-14-3	-6,04	9,18E-10	398	3,7E-04	-3,44	0,20	3,44	58
328	139158-26-2	-5,90	1,25E-09	326	4,1E-04	-3,39	1,50	3,39	59
338	141079-02-9	-5,94	1,14E-09	373	4,3E-04	-3,37	0,40	3,37	60
230	081518-29-8	-5,85	1,41E-09	302	4,3E-04	-3,37	0,00	3,37	61
113	034771-66-9	-5,97	1,08E-09	404	4,4E-04	-3,36	0,57	3,36	62
287	098532-72-0	-5,93	1,16E-09	377	4,4E-04	-3,36	0,97	3,36	63
268	098519-30-3	-5,93	1,17E-09	377	4,4E-04	-3,35	0,97	3,35	64
333	141078-94-6	-5,91	1,24E-09	359	4,4E-04	-3,35	0,40	3,35	65
245	086598-92-7	-5,96	1,09E-09	412	4,5E-04	-3,35	0,20	3,35	66
90	019794-93-5	-5,91	1,22E-09	372	4,5E-04	-3,34	0,77	3,34	67
141	043029-44-3	-5,76	1,74E-09	278	4,8E-04	-3,31	0,20	3,31	68
364	422556-08-9	-5,95	1,13E-09	434	4,9E-04	-3,31	0,20	3,31	69
51	003864-99-1	-5,86	1,38E-09	358	4,9E-04	-3,31	1,13	3,31	70
186	066535-86-2	-5,71	1,93E-09	280	5,4E-04	-3,27	0,57	3,27	71
313	119446-68-3	-5,87	1,35E-09	406	5,5E-04	-3,26	0,00	3,26	72
303	103922-48-1	-5,81	1,54E-09	358	5,5E-04	-3,26	1,13	3,26	73
252	097232-75-2	-5,85	1,40E-09	425	6,0E-04	-3,23	0,73	3,23	74
94	023711-34-4	-5,67	2,15E-09	288	6,2E-04	-3,21	0,40	3,21	75
238	083044-91-1	-5,80	1,60E-09	388	6,2E-04	-3,21	0,17	3,21	76
50	003846-71-7	-5,69	2,02E-09	323	6,5E-04	-3,19	0,93	3,19	77
242	085509-19-9	-5,68	2,09E-09	315	6,6E-04	-3,18	0,20	3,18	78
274	098519-37-0	-5,75	1,78E-09	370	6,6E-04	-3,18	1,13	3,18	79
327	139158-25-1	-5,59	2,59E-09	263	6,8E-04	-3,17	1,13	3,17	80
233	081518-37-8	-5,68	2,11E-09	325	6,9E-04	-3,16	0,20	3,16	81
167	057801-94-2	-5,80	1,58E-09	438	6,9E-04	-3,16	0,93	3,16	82
117	036437-37-3	-5,66	2,18E-09	323	7,0E-04	-3,15	1,13	3,15	83
355	147150-35-4	-5,78	1,64E-09	430	7,1E-04	-3,15	0,37	3,15	84
362	317815-83-1	-5,73	1,87E-09	390	7,3E-04	-3,14	0,57	3,14	85
234	081518-41-4	-5,73	1,88E-09	390	7,3E-04	-3,13	0,00	3,13	86
258	098519-02-9	-5,75	1,79E-09	412	7,4E-04	-3,13	0,00	3,13	87
276	098519-41-6	-5,74	1,82E-09	412	7,5E-04	-3,12	0,17	3,12	88
34	003147-75-9	-5,62	2,41E-09	323	7,8E-04	-3,11	0,93	3,11	89
158	055179-31-2	-5,64	2,31E-09	337	7,8E-04	-3,11	0,00	3,11	90
161	055425-38-2	-5,55	2,81E-09	288	8,1E-04	-3,09	0,57	3,09	91
380	XXX017	-5,64	2,29E-09	354	8,1E-04	-3,09	0,00	3,09	92
381	XXX018	-5,64	2,31E-09	351	8,1E-04	-3,09	0,00	3,09	93
384	XXX021	-5,64	2,30E-09	354	8,1E-04	-3,09	0,00	3,09	94
300	103112-35-2	-5,69	2,02E-09	403	8,2E-04	-3,09	0,57	3,09	95
103	028911-01-5	-5,61	2,44E-09	343	8,4E-04	-3,08	0,73	3,08	96
310	114369-43-6	-5,58	2,60E-09	337	8,8E-04	-3,06	0,00	3,06	97
243	085634-51-1	-5,58	2,62E-09	339	8,9E-04	-3,05	1,13	3,05	98
23	001326-66-5	-5,56	2,75E-09	327	9,0E-04	-3,05	0,40	3,05	99
312	119126-15-7	-5,70	1,99E-09	461	9,2E-04	-3,04	0,57	3,04	100

360	212201-70-2	-5,66	2,20E-09	427	9,4E-04	-3,03	0,17	3,03	101
119	037160-06-8	-5,45	3,52E-09	273	9,6E-04	-3,02	0,20	3,02	102
166	057801-81-7	-5,61	2,44E-09	394	9,6E-04	-3,02	0,73	3,02	103
352	145026-81-9	-5,61	2,45E-09	398	9,7E-04	-3,01	0,37	3,01	104
171	059338-93-1	-5,51	3,11E-09	315	9,8E-04	-3,01	0,20	3,01	105
361	219714-96-2	-5,68	2,10E-09	483	1,0E-03	-2,99	0,93	2,99	106
288	098532-73-1	-5,59	2,58E-09	398	1,0E-03	-2,99	0,00	2,99	107
122	040054-69-1	-5,52	3,02E-09	343	1,0E-03	-2,99	0,53	2,99	108
329	139528-85-1	-5,58	2,63E-09	418	1,1E-03	-2,96	0,17	2,96	109
318	125306-83-4	-5,49	3,22E-09	350	1,1E-03	-2,95	0,00	2,95	110
157	054123-06-7	-5,48	3,35E-09	349	1,2E-03	-2,93	0,73	2,93	111
363	348635-87-0	-5,59	2,59E-09	466	1,2E-03	-2,92	0,53	2,92	112
83	015497-45-7	-5,33	4,67E-09	260	1,2E-03	-2,92	0,73	2,92	113
272	098519-34-7	-5,47	3,42E-09	355	1,2E-03	-2,92	0,60	2,92	114
292	098532-80-0	-5,47	3,42E-09	355	1,2E-03	-2,92	0,60	2,92	115
271	098519-33-6	-5,44	3,59E-09	341	1,2E-03	-2,91	0,00	2,91	116
150	054028-89-6	-5,40	3,96E-09	311	1,2E-03	-2,91	0,53	2,91	117
145	054028-81-8	-5,40	3,96E-09	311	1,2E-03	-2,91	0,53	2,91	118
146	054028-83-0	-5,44	3,63E-09	341	1,2E-03	-2,91	0,53	2,91	119
326	139158-24-0	-5,30	5,00E-09	249	1,2E-03	-2,90	1,13	2,90	120
151	054028-90-9	-5,43	3,68E-09	341	1,3E-03	-2,90	0,53	2,90	121
316	125225-28-7	-5,41	3,88E-09	334	1,3E-03	-2,89	0,00	2,89	122
293	098532-81-1	-5,41	3,86E-09	341	1,3E-03	-2,88	0,00	2,88	123
104	028981-97-7	-5,36	4,39E-09	309	1,4E-03	-2,87	0,53	2,87	124
353	145701-21-9	-5,47	3,38E-09	406	1,4E-03	-2,86	0,37	2,86	125
376	XXX013	-5,42	3,80E-09	366	1,4E-03	-2,86	0,20	2,86	126
173	060207-90-1	-5,39	4,07E-09	342	1,4E-03	-2,86	0,20	2,86	127
152	054028-91-0	-5,28	5,23E-09	276	1,4E-03	-2,84	0,73	2,84	128
291	098532-77-5	-5,42	3,80E-09	382	1,5E-03	-2,84	0,00	2,84	129
285	098532-70-8	-5,41	3,86E-09	384	1,5E-03	-2,83	0,00	2,83	130
164	056396-43-1	-5,35	4,49E-09	332	1,5E-03	-2,83	0,00	2,83	131
205	078218-53-8	-5,32	4,75E-09	316	1,5E-03	-2,82	1,10	2,82	132
52	003896-11-5	-5,32	4,84E-09	316	1,5E-03	-2,82	0,37	2,82	133
184	066246-88-6	-5,27	5,39E-09	284	1,5E-03	-2,82	0,00	2,82	134
115	036325-69-6	-5,28	5,22E-09	294	1,5E-03	-2,81	0,20	2,81	135
366	XXX002	-5,48	3,34E-09	469	1,6E-03	-2,81	1,10	2,81	136
296	098532-85-5	-5,35	4,43E-09	355	1,6E-03	-2,80	0,97	2,80	137
148	054028-85-2	-5,30	5,03E-09	317	1,6E-03	-2,80	0,73	2,80	138
154	054028-93-2	-5,28	5,24E-09	306	1,6E-03	-2,79	0,53	2,79	139
153	054028-92-1	-5,28	5,31E-09	306	1,6E-03	-2,79	0,53	2,79	140
155	054028-94-3	-5,20	6,27E-09	262	1,6E-03	-2,78	0,73	2,78	141
314	122836-35-5	-5,37	4,26E-09	387	1,6E-03	-2,78	0,17	2,78	142
107	029975-16-4	-5,25	5,59E-09	295	1,6E-03	-2,78	0,73	2,78	143
190	068049-83-2	-5,30	5,00E-09	338	1,7E-03	-2,77	0,40	2,77	144
257	098519-01-8	-5,34	4,60E-09	368	1,7E-03	-2,77	0,00	2,77	145

88	018811-70-6	-5,17	6,70E-09	253	1,7E-03	-2,77	0,40	2,77	146
286	098532-71-9	-5,29	5,16E-09	329	1,7E-03	-2,77	0,00	2,77	147
270	098519-32-5	-5,28	5,19E-09	327	1,7E-03	-2,77	0,00	2,77	148
267	098519-29-0	-5,29	5,18E-09	329	1,7E-03	-2,77	0,00	2,77	149
269	098519-31-4	-5,28	5,20E-09	329	1,7E-03	-2,77	0,00	2,77	150
294	098532-82-2	-5,28	5,27E-09	327	1,7E-03	-2,76	0,00	2,76	151
273	098519-35-8	-5,28	5,28E-09	327	1,7E-03	-2,76	0,00	2,76	152
315	125116-23-6	-5,26	5,50E-09	320	1,8E-03	-2,75	0,00	2,75	153
356	147993-59-7	-5,31	4,86E-09	363	1,8E-03	-2,75	1,17	2,75	154
156	054028-95-4	-5,24	5,78E-09	306	1,8E-03	-2,75	0,53	2,75	155
371	XXX008	-5,31	4,94E-09	358	1,8E-03	-2,75	0,00	2,75	156
374	XXX011	-5,27	5,41E-09	334	1,8E-03	-2,74	0,00	2,74	157
305	106325-08-0	-5,25	5,60E-09	330	1,8E-03	-2,73	0,00	2,73	158
321	128639-02-1	-5,34	4,53E-09	412	1,9E-03	-2,73	0,00	2,73	159
385	XXX022	-5,33	4,73E-09	404	1,9E-03	-2,72	0,53	2,72	160
264	098519-25-6	-5,28	5,20E-09	368	1,9E-03	-2,72	0,00	2,72	161
375	XXX012	-5,24	5,73E-09	334	1,9E-03	-2,72	0,00	2,72	162
367	XXX003	-5,24	5,79E-09	334	1,9E-03	-2,71	0,00	2,71	163
281	098532-66-2	-5,28	5,27E-09	368	1,9E-03	-2,71	0,00	2,71	164
44	003652-27-5	-5,12	7,67E-09	255	2,0E-03	-2,71	0,20	2,71	165
324	131983-72-7	-5,21	6,22E-09	318	2,0E-03	-2,70	0,00	2,70	166
200	078149-96-9	-5,18	6,56E-09	303	2,0E-03	-2,70	0,90	2,70	167
311	116255-48-2	-5,27	5,36E-09	377	2,0E-03	-2,69	0,00	2,69	168
306	107534-96-3	-5,18	6,56E-09	308	2,0E-03	-2,69	0,00	2,69	169
365	865318-97-4	-5,13	7,47E-09	275	2,1E-03	-2,69	0,93	2,69	170
325	136426-54-5	-5,26	5,48E-09	376	2,1E-03	-2,69	0,17	2,69	171
35	003147-76-0	-5,11	7,78E-09	267	2,1E-03	-2,68	0,37	2,68	172
228	081518-27-6	-5,22	6,05E-09	348	2,1E-03	-2,68	0,20	2,68	173
359	178928-70-6	-5,21	6,18E-09	344	2,1E-03	-2,67	0,17	2,67	174
208	078218-56-1	-5,17	6,74E-09	320	2,2E-03	-2,67	0,17	2,67	175
284	098532-69-5	-5,18	6,64E-09	331	2,2E-03	-2,66	0,00	2,66	176
84	015805-10-4	-5,06	8,80E-09	250	2,2E-03	-2,66	0,57	2,66	177
174	060207-93-4	-5,16	6,91E-09	328	2,3E-03	-2,64	0,00	2,64	178
261	098519-06-3	-5,17	6,80E-09	334	2,3E-03	-2,64	0,00	2,64	179
290	098532-75-3	-5,14	7,28E-09	313	2,3E-03	-2,64	0,00	2,64	180
277	098519-43-8	-5,17	6,84E-09	334	2,3E-03	-2,64	0,00	2,64	181
229	081518-28-7	-5,09	8,12E-09	283	2,3E-03	-2,64	0,20	2,64	182
212	078218-60-7	-5,07	8,47E-09	274	2,3E-03	-2,63	1,07	2,63	183
283	098532-68-4	-5,18	6,67E-09	354	2,4E-03	-2,63	0,00	2,63	184
265	098519-26-7	-5,17	6,76E-09	354	2,4E-03	-2,62	0,00	2,62	185
299	099793-75-6	-5,16	6,88E-09	352	2,4E-03	-2,62	0,00	2,62	186
279	098532-64-0	-5,11	7,70E-09	315	2,4E-03	-2,62	0,00	2,62	187
266	098519-28-9	-5,11	7,71E-09	315	2,4E-03	-2,61	0,00	2,61	188
256	098519-00-7	-5,15	7,11E-09	354	2,5E-03	-2,60	0,00	2,60	189
109	031409-18-4	-5,00	9,99E-09	254	2,5E-03	-2,60	0,00	2,60	190

301	103112-36-3	-5,16	6,88E-09	375	2,6E-03	-2,59	0,73	2,59	191
227	081518-26-5	-5,00	1,01E-08	269	2,7E-03	-2,57	0,40	2,57	192
307	112143-82-5	-5,05	8,90E-09	314	2,8E-03	-2,55	0,57	2,55	193
372	XXX009	-5,05	8,95E-09	312	2,8E-03	-2,55	0,00	2,55	194
64	006054-53-1	-4,95	1,13E-08	248	2,8E-03	-2,55	0,00	2,55	195
195	075736-33-3	-5,07	8,55E-09	328	2,8E-03	-2,55	0,00	2,55	196
259	098519-04-1	-5,05	8,89E-09	320	2,8E-03	-2,55	0,00	2,55	197
357	149508-90-7	-5,01	9,71E-09	293	2,8E-03	-2,55	0,20	2,55	198
207	078218-55-0	-5,05	8,90E-09	324	2,9E-03	-2,54	0,37	2,54	199
383	XXX020	-5,09	8,20E-09	358	2,9E-03	-2,53	0,20	2,53	200
262	098519-07-4	-5,03	9,24E-09	320	3,0E-03	-2,53	0,00	2,53	201
241	083657-24-3	-5,04	9,09E-09	326	3,0E-03	-2,53	0,00	2,53	202
246	088671-89-0	-4,99	1,03E-08	289	3,0E-03	-2,53	0,00	2,53	203
214	078218-65-2	-4,92	1,20E-08	248	3,0E-03	-2,52	0,73	2,52	204
275	098519-39-2	-5,00	1,01E-08	299	3,0E-03	-2,52	0,00	2,52	205
43	003652-25-3	-4,86	1,38E-08	218	3,0E-03	-2,52	0,20	2,52	206
255	098518-99-1	-4,99	1,02E-08	299	3,0E-03	-2,52	0,00	2,52	207
282	098532-67-3	-5,05	8,94E-09	340	3,0E-03	-2,52	0,00	2,52	208
221	079983-71-4	-5,01	9,69E-09	314	3,0E-03	-2,52	0,00	2,52	209
295	098532-83-3	-4,98	1,05E-08	299	3,1E-03	-2,51	0,00	2,51	210
386	XXX023	-4,97	1,08E-08	292	3,2E-03	-2,50	0,00	2,50	211
370	XXX007	-4,98	1,05E-08	304	3,2E-03	-2,49	0,00	2,49	212
240	083657-17-4	-4,92	1,20E-08	292	3,5E-03	-2,45	0,00	2,45	213
260	098519-05-2	-4,94	1,16E-08	306	3,5E-03	-2,45	0,00	2,45	214
289	098532-74-2	-4,92	1,21E-08	299	3,6E-03	-2,44	0,00	2,44	215
263	098519-24-5	-4,89	1,29E-08	285	3,7E-03	-2,44	0,00	2,44	216
278	098519-49-4	-4,89	1,29E-08	285	3,7E-03	-2,43	0,00	2,43	217
198	076738-62-0	-4,90	1,27E-08	294	3,7E-03	-2,43	0,00	2,43	218
250	094361-06-5	-4,89	1,29E-08	292	3,8E-03	-2,43	0,00	2,43	219
19	001028-08-6	-4,88	1,32E-08	288	3,8E-03	-2,42	0,73	2,42	220
203	078218-51-6	-4,93	1,16E-08	329	3,8E-03	-2,42	0,17	2,42	221
149	054028-86-3	-4,89	1,29E-08	301	3,9E-03	-2,41	0,53	2,41	222
42	003652-23-1	-4,75	1,77E-08	220	3,9E-03	-2,41	0,20	2,41	223
253	098518-95-7	-4,86	1,38E-08	285	3,9E-03	-2,40	0,00	2,40	224
373	XXX010	-4,98	1,04E-08	384	4,0E-03	-2,40	0,20	2,40	225
322	129586-32-9	-4,86	1,37E-08	292	4,0E-03	-2,40	0,20	2,40	226
218	078371-73-0	-4,91	1,23E-08	332	4,1E-03	-2,39	0,90	2,39	227
188	067465-03-6	-4,76	1,75E-08	234	4,1E-03	-2,39	0,00	2,39	228
142	043121-43-3	-4,85	1,42E-08	294	4,2E-03	-2,38	0,00	2,38	229
172	060207-31-0	-4,84	1,44E-08	300	4,3E-03	-2,36	0,00	2,36	230
159	055219-65-3	-4,83	1,49E-08	296	4,4E-03	-2,35	0,00	2,35	231
297	098967-40-9	-4,85	1,40E-08	325	4,5E-03	-2,34	0,37	2,34	232
354	145701-23-1	-4,90	1,27E-08	359	4,5E-03	-2,34	0,53	2,34	233
280	098532-65-1	-4,76	1,73E-08	271	4,7E-03	-2,33	0,20	2,33	234
197	076674-21-0	-4,81	1,56E-08	301	4,7E-03	-2,33	0,00	2,33	235

216	078324-76-2	-4,72	1,88E-08	249	4,7E-03	-2,33	1,10	2,33	236
368	XXX004	-4,94	1,14E-08	416	4,7E-03	-2,33	0,77	2,33	237
127	041735-38-0	-4,79	1,63E-08	293	4,8E-03	-2,32	0,00	2,32	238
132	041735-50-6	-4,79	1,63E-08	293	4,8E-03	-2,32	0,00	2,32	239
168	059026-08-3	-4,78	1,67E-08	288	4,8E-03	-2,32	0,57	2,32	240
182	066104-34-5	-4,70	2,00E-08	241	4,8E-03	-2,32	0,00	2,32	241
63	005873-30-3	-4,71	1,97E-08	247	4,9E-03	-2,31	0,17	2,31	242
112	032723-50-5	-4,66	2,18E-08	224	4,9E-03	-2,31	0,00	2,31	243
235	082200-72-4	-4,78	1,66E-08	296	4,9E-03	-2,31	0,00	2,31	244
247	089482-17-7	-4,78	1,66E-08	296	4,9E-03	-2,31	0,00	2,31	245
196	076608-88-3	-4,72	1,93E-08	257	5,0E-03	-2,31	0,00	2,31	246
308	112281-77-3	-4,87	1,33E-08	372	5,0E-03	-2,30	0,00	2,30	247
179	063870-37-1	-4,70	2,00E-08	249	5,0E-03	-2,30	0,37	2,30	248
160	055375-40-1	-4,72	1,91E-08	263	5,0E-03	-2,30	0,37	2,30	249
183	066104-44-7	-4,67	2,14E-08	239	5,1E-03	-2,29	0,17	2,29	250
181	064082-38-8	-4,77	1,68E-08	306	5,1E-03	-2,29	0,37	2,29	251
102	028401-89-0	-4,67	2,13E-08	243	5,2E-03	-2,29	0,17	2,29	252
41	003652-22-0	-4,60	2,52E-08	206	5,2E-03	-2,28	0,20	2,28	253
192	070292-10-3	-4,67	2,13E-08	253	5,4E-03	-2,27	0,00	2,27	254
201	078150-00-2	-4,71	1,93E-08	280	5,4E-03	-2,27	0,37	2,27	255
244	086386-73-4	-4,75	1,77E-08	306	5,4E-03	-2,27	0,00	2,27	256
248	089786-04-9	-4,73	1,86E-08	298	5,5E-03	-2,26	0,20	2,26	257
377	XXX014	-4,79	1,63E-08	345	5,6E-03	-2,25	0,33	2,25	258
202	078150-02-4	-4,68	2,10E-08	274	5,7E-03	-2,24	0,53	2,24	259
254	098518-96-8	-4,67	2,12E-08	273	5,8E-03	-2,24	0,17	2,24	260
20	001031-47-6	-4,71	1,97E-08	294	5,8E-03	-2,24	0,17	2,24	261
28	001704-66-1	-4,60	2,50E-08	237	5,9E-03	-2,23	0,00	2,23	262
189	067465-05-8	-4,56	2,74E-08	217	5,9E-03	-2,23	0,00	2,23	263
130	041735-44-8	-4,68	2,08E-08	293	6,1E-03	-2,21	0,00	2,21	264
382	XXX019	-4,72	1,92E-08	322	6,2E-03	-2,21	0,53	2,21	265
126	041735-30-2	-4,63	2,33E-08	268	6,2E-03	-2,21	0,00	2,21	266
60	005369-84-6	-4,56	2,74E-08	232	6,4E-03	-2,20	0,00	2,20	267
220	078592-90-2	-4,63	2,35E-08	274	6,4E-03	-2,19	0,73	2,19	268
100	027210-18-0	-4,55	2,84E-08	229	6,5E-03	-2,19	0,17	2,19	269
48	003683-95-2	-4,63	2,36E-08	277	6,5E-03	-2,18	0,90	2,18	270
139	041834-21-3	-4,59	2,58E-08	256	6,6E-03	-2,18	0,57	2,18	271
30	002440-22-4	-4,53	2,98E-08	225	6,7E-03	-2,17	0,20	2,17	272
111	032362-89-3	-4,42	3,82E-08	178	6,8E-03	-2,17	0,37	2,17	273
45	003652-31-1	-4,44	3,62E-08	192	6,9E-03	-2,16	0,00	2,16	274
180	064057-50-7	-4,61	2,44E-08	286	7,0E-03	-2,16	0,00	2,16	275
210	078218-58-3	-4,57	2,68E-08	266	7,1E-03	-2,15	0,37	2,15	276
74	010187-79-8	-4,54	2,89E-08	251	7,3E-03	-2,14	0,00	2,14	277
191	069141-50-0	-4,57	2,72E-08	267	7,3E-03	-2,14	0,57	2,14	278
369	XXX006	-4,57	2,67E-08	272	7,3E-03	-2,14	0,00	2,14	279
125	041735-29-9	-4,54	2,89E-08	252	7,3E-03	-2,14	0,00	2,14	280

136	041735-56-2	-4,56	2,75E-08	272	7,5E-03	-2,13	0,20	2,13	281
135	041735-55-1	-4,50	3,18E-08	240	7,6E-03	-2,12	0,00	2,12	282
163	056383-11-0	-4,46	3,49E-08	219	7,6E-03	-2,12	0,00	2,12	283
162	056383-06-3	-4,45	3,51E-08	219	7,7E-03	-2,11	0,00	2,11	284
99	027022-50-0	-4,49	3,26E-08	237	7,7E-03	-2,11	0,53	2,11	285
79	013257-88-0	-4,25	5,58E-08	141	7,9E-03	-2,10	0,57	2,10	286
185	066492-64-6	-4,48	3,34E-08	236	7,9E-03	-2,10	0,00	2,10	287
199	077314-77-3	-4,41	3,90E-08	204	8,0E-03	-2,10	0,00	2,10	288
215	078218-66-3	-4,46	3,44E-08	234	8,0E-03	-2,09	0,53	2,09	289
211	078218-59-4	-4,51	3,11E-08	260	8,1E-03	-2,09	0,53	2,09	290
120	038942-51-7	-4,37	4,29E-08	191	8,2E-03	-2,09	0,00	2,09	291
49	003770-47-6	-4,36	4,32E-08	192	8,3E-03	-2,08	0,00	2,08	292
77	010187-89-0	-4,43	3,74E-08	223	8,3E-03	-2,08	0,00	2,08	293
61	005472-71-9	-4,41	3,85E-08	218	8,4E-03	-2,08	0,00	2,08	294
47	003663-24-9	-4,32	4,81E-08	175	8,4E-03	-2,08	0,77	2,08	295
82	015421-84-8	-4,38	4,17E-08	205	8,6E-03	-2,07	0,17	2,07	296
206	078218-54-9	-4,48	3,29E-08	260	8,6E-03	-2,07	0,73	2,07	297
46	003652-32-2	-4,35	4,46E-08	192	8,6E-03	-2,07	0,00	2,07	298
219	078371-74-1	-4,55	2,83E-08	304	8,6E-03	-2,07	0,73	2,07	299
194	075020-35-8	-4,40	4,03E-08	215	8,7E-03	-2,06	0,17	2,06	300
116	036411-52-6	-4,37	4,31E-08	204	8,8E-03	-2,06	0,00	2,06	301
223	080584-88-9	-4,43	3,71E-08	250	9,3E-03	-2,03	0,17	2,03	302
37	003310-68-7	-4,27	5,43E-08	175	9,5E-03	-2,02	0,20	2,02	303
224	080584-89-0	-4,41	3,86E-08	250	9,6E-03	-2,02	0,17	2,02	304
124	041735-28-8	-4,39	4,08E-08	238	9,7E-03	-2,01	0,00	2,01	305
110	031701-42-5	-4,36	4,36E-08	225	9,8E-03	-2,01	0,20	2,01	306
133	041735-51-7	-4,39	4,03E-08	254	1,0E-02	-1,99	0,20	1,99	307
128	041735-41-5	-4,33	4,69E-08	223	1,0E-02	-1,98	0,00	1,98	308
138	041814-78-2	-4,25	5,61E-08	189	1,1E-02	-1,97	0,37	1,97	309
27	001680-44-0	-4,12	7,53E-08	145	1,1E-02	-1,96	0,40	1,96	310
176	061691-97-2	-4,33	4,69E-08	236	1,1E-02	-1,96	0,17	1,96	311
17	000947-85-3	-4,26	5,46E-08	203	1,1E-02	-1,96	0,00	1,96	312
75	010187-84-5	-4,26	5,55E-08	209	1,2E-02	-1,94	0,00	1,94	313
71	007411-23-6	-4,29	5,12E-08	227	1,2E-02	-1,93	0,90	1,93	314
93	023633-05-8	-4,31	4,91E-08	239	1,2E-02	-1,93	0,00	1,93	315
59	005302-27-2	-4,18	6,57E-08	185	1,2E-02	-1,92	0,53	1,92	316
204	078218-52-7	-4,30	4,99E-08	246	1,2E-02	-1,91	0,53	1,91	317
217	078371-72-9	-4,37	4,29E-08	290	1,2E-02	-1,90	0,73	1,90	318
114	035515-45-8	-4,28	5,22E-08	239	1,2E-02	-1,90	0,17	1,90	319
169	059338-86-2	-4,20	6,28E-08	207	1,3E-02	-1,89	0,17	1,89	320
14	000939-07-1	-4,09	8,13E-08	161	1,3E-02	-1,88	0,17	1,88	321
131	041735-45-9	-4,23	5,88E-08	223	1,3E-02	-1,88	0,00	1,88	322
96	024054-57-7	-4,26	5,52E-08	239	1,3E-02	-1,88	0,00	1,88	323
16	000944-91-2	-4,14	7,23E-08	189	1,4E-02	-1,86	0,00	1,86	324
56	004368-68-7	-4,06	8,61E-08	159	1,4E-02	-1,86	0,00	1,86	325

134	041735-54-0	-4,17	6,70E-08	215	1,4E-02	-1,84	0,20	1,84	326
323	129909-90-6	-4,22	6,00E-08	241	1,4E-02	-1,84	0,93	1,84	327
379	XXX016	-4,32	4,82E-08	304	1,5E-02	-1,83	0,37	1,83	328
137	041735-57-3	-4,18	6,55E-08	224	1,5E-02	-1,83	0,00	1,83	329
65	006085-94-5	-4,03	9,32E-08	159	1,5E-02	-1,83	0,00	1,83	330
39	003357-42-4	-3,97	1,06E-07	145	1,5E-02	-1,81	0,20	1,81	331
26	001600-61-9	-4,13	7,42E-08	210	1,6E-02	-1,81	0,00	1,81	332
53	004184-79-6	-3,96	1,09E-07	147	1,6E-02	-1,80	0,37	1,80	333
15	000939-08-2	-4,00	9,89E-08	162	1,6E-02	-1,80	0,00	1,80	334
7	000136-85-6	-3,91	1,23E-07	133	1,6E-02	-1,79	0,40	1,79	335
170	059338-92-0	-4,05	8,85E-08	193	1,7E-02	-1,77	0,00	1,77	336
81	014803-99-7	-3,92	1,21E-07	146	1,8E-02	-1,75	0,00	1,75	337
76	010187-86-7	-4,07	8,51E-08	209	1,8E-02	-1,75	0,00	1,75	338
2	000094-97-3	-3,93	1,19E-07	154	1,8E-02	-1,74	0,20	1,74	339
101	027799-91-3	-3,91	1,23E-07	149	1,8E-02	-1,74	0,00	1,74	340
86	016584-05-7	-3,90	1,25E-07	147	1,8E-02	-1,73	0,00	1,73	341
92	021532-04-7	-3,98	1,06E-07	176	1,9E-02	-1,73	0,57	1,73	342
67	006789-99-7	-3,81	1,53E-07	123	1,9E-02	-1,72	0,97	1,72	343
106	029878-31-7	-3,84	1,43E-07	133	1,9E-02	-1,72	0,20	1,72	344
175	060932-58-3	-3,93	1,17E-07	163	1,9E-02	-1,72	0,20	1,72	345
3	000095-14-7	-3,79	1,61E-07	119	1,9E-02	-1,72	0,40	1,72	346
9	000288-36-8	-3,56	2,77E-07	69,1	1,9E-02	-1,72	1,53	1,72	347
13	000938-56-7	-3,92	1,19E-07	163	1,9E-02	-1,71	0,00	1,71	348
78	013091-80-0	-4,01	9,76E-08	199	1,9E-02	-1,71	0,37	1,71	349
72	007532-52-7	-3,99	1,03E-07	195	2,0E-02	-1,70	0,20	1,70	350
378	XXX015	-4,18	6,61E-08	304	2,0E-02	-1,70	0,17	1,70	351
8	000273-40-5	-3,76	1,72E-07	121	2,1E-02	-1,68	0,77	1,68	352
129	041735-42-6	-4,00	1,01E-07	209	2,1E-02	-1,68	0,00	1,68	353
29	002338-12-7	-3,89	1,30E-07	164	2,1E-02	-1,67	0,20	1,67	354
209	078218-57-2	-4,04	9,19E-08	232	2,1E-02	-1,67	0,53	1,67	355
66	006299-39-4	-3,88	1,32E-07	164	2,2E-02	-1,67	0,20	1,67	356
80	013351-73-0	-3,79	1,63E-07	133	2,2E-02	-1,66	0,40	1,66	357
68	006818-99-1	-3,68	2,09E-07	104	2,2E-02	-1,66	1,10	1,66	358
70	007170-01-6	-3,57	2,71E-07	83,1	2,3E-02	-1,65	1,13	1,65	359
10	000288-88-0	-3,49	3,26E-07	69,1	2,3E-02	-1,65	1,33	1,65	360
21	001123-54-2	-3,78	1,66E-07	136	2,3E-02	-1,65	0,40	1,65	361
304	104958-85-2	-3,97	1,07E-07	215	2,3E-02	-1,64	0,20	1,64	362
6	000134-58-7	-3,82	1,52E-07	152	2,3E-02	-1,64	0,97	1,64	363
87	018076-61-4	-3,76	1,74E-07	134	2,3E-02	-1,63	0,40	1,63	364
32	002683-90-1	-3,76	1,73E-07	137	2,4E-02	-1,63	0,60	1,63	365
91	021050-95-3	-3,80	1,59E-07	154	2,4E-02	-1,61	0,37	1,61	366
251	094667-47-7	-3,91	1,23E-07	199	2,5E-02	-1,61	0,57	1,61	367
40	003641-10-9	-3,56	2,78E-07	94,1	2,6E-02	-1,58	0,53	1,58	368
25	001468-26-4	-3,75	1,78E-07	153	2,7E-02	-1,57	0,77	1,57	369
1	000061-82-5	-3,49	3,26E-07	84,1	2,7E-02	-1,56	0,97	1,56	370

31	002592-95-2	-3,69	2,04E-07	135	2,8E-02	-1,56	0,20	1,56	371
118	036791-04-5	-3,95	1,13E-07	244	2,8E-02	-1,56	0,97	1,56	372
144	053817-16-6	-3,62	2,37E-07	119	2,8E-02	-1,55	0,37	1,55	373
55	004343-73-1	-3,72	1,90E-07	155	2,9E-02	-1,53	0,20	1,53	374
165	056881-36-8	-3,70	2,00E-07	150	3,0E-02	-1,52	0,20	1,52	375
11	000584-13-4	-3,44	3,62E-07	84,1	3,0E-02	-1,52	1,10	1,52	376
105	029440-31-1	-3,85	1,43E-07	218	3,1E-02	-1,51	0,53	1,51	377
121	039968-33-7	-3,63	2,35E-07	136	3,2E-02	-1,50	0,20	1,50	378
73	010109-05-4	-3,65	2,22E-07	156	3,5E-02	-1,46	0,40	1,46	379
36	003232-84-6	-3,42	3,84E-07	101	3,9E-02	-1,41	1,47	1,41	380
24	001455-77-2	-3,38	4,21E-07	99,1	4,2E-02	-1,38	0,73	1,38	381
58	004928-88-5	-3,47	3,38E-07	127	4,3E-02	-1,37	0,20	1,37	382
57	004928-87-4	-3,37	4,29E-07	113	4,8E-02	-1,31	0,37	1,31	383
98	026621-45-4	-3,38	4,14E-07	128	5,3E-02	-1,28	0,37	1,28	384
54	004314-22-1	-3,38	4,17E-07	127	5,3E-02	-1,28	0,53	1,28	385
12	000932-64-9	-3,23	5,83E-07	130	7,6E-02	-1,12	0,90	1,12	386

Appendix 5

Case study 5: Prioritization based on risk assessment of BTAZs

Overview

This case study describes a ranking based on risk assessments based on a unit emission scenario. Predictive distributions for QSARs were used as sources in uncertainty analyis (Golsteijn, Iqbal et al. in review). The ranking of BTAZs was done based on assessment of PNEC and PEC given a unit emission. As PEC-values are proportional to emission, the ratio between PNEC/PEC (i.e. the reciprocal of the Risk Characterization ratio = PEC/PNEC) can be interpreted as the Maximum Permissible Emission (MPE). A measure of Relative Risk (RR) was dervied from the expectation E(MPE) or x^{th} percentile MPE(x^{th}) according to:

$$RR(E) = -\log E(MPE) \quad \text{and} \quad RR(x^{th}) = -\log MPE(x^{th}),$$

respectively. Ranking based on expectations is approriate when we are indifferent to the existence of high and low values and only base decision on what to expect in average (risk neutral). Relative risk based on the xth percentile implement different degrees of cautioness (given that $x < 50$) in the prioritization. A log tranformation was performed to make the data more evenly distributed.

Mapping Relative Risk to chemical domain

A Partial Least Sqares (PSL) regression was made using dragon descriptors (2 dim 6.0) to find the directions in chemical domain that best explained differences in relative risk of the 8 selected compounds (Table 1). Due to the very small data set, the PLS could not be validated and is only used here to illustrate the approach. The pattern seen by plotting the first and second PLS component (Figure 1 and 2) shows trends in relative risk. The most influenting molecular descriptors can be used to explain differences in relative risk. Ranking based on conservative values resulted in a larger difference between low and high values and a stronger PLS model.

Evaluating predictive reliability

The PEC and PNEC assessments were based on several QSARs to predict input parameters of physico-chemical properties or activities. Input parameters do more or less influence the final outcome of an assessment. Uncertainty in QSAR predicted input parameters was quantified as probability distributions describing the variation in a prediction that can be derived by statistical principles.

These uncertainties had been propagated through the assessment by Monte Carlo simulation and contributed to the total uncertainty in the output (MPE). Sensitivity analysis showed differences in the relative contribution to uncertainty from different QSARs.

The treatment of uncertainty described so far considers a quantitative uncertainty in a QSAR prediction, i.e. related to the magnitude of the error between having an experimentally based estimate and a QSAR prediction. Uncertainty in a QSAR prediction is also qualitative and depends for example on the degree of extrapolation. Confidence in a QSAR prediction is lower when the query compound falls out of a QSARs domain of applicability. Now, given differences in the final outputs sensitivities to QSAR predicted input parameters, the influence of lower confidence in individual predictions may be more or less relevant to the overall confidence in an assessment. In order to evaluate the quality of measures of RR we needed a way to propagate the possible lower confidence in individual QSAR predictions, as seen when a compound is out of the applicability domain. Here we show the results when a lower confidence due to a compound being out of the applicability domain was considered by enlarging quantitative uncertainty in the corresponding QSAR prediction. Compounds were judged less reliable when their hat value exceeded the chosen cut-off. The difference in RR based on the original assessment and the extended assessment show the relative contribution of lower predictive reliability. The sizes of the triangles in the Figures reflect the difference between the 95th percentiles of the RR's in percent. Among the eight compounds only one (Flupoxam) was out of the applicability domain in several of the QSARs. Flupoxam and Sulfentrazone were the two compounds with the highest relative risk.

Final ranking

The descriptor informed Relative Risk scheme was applied on 386 BTAZs (Cassini, Kovarich et al. in review). The eight training assessments were spread out over the range of Relative Risks (Figure 3). The assessment with low predictive reliability did not constitute an extreme value. The rankings of the 386 BTAZs are found in Table 2 below.

References

Cassini, S., S. Kovarich, et al. (in review). "Evaluation of CADASTER QSAR models for aquatic toxicity of (benzo-)triazoles and prioritization by consensus."

Golsteijn, L., M. S. Iqbal, et al. (in review). "The Relative Importance of Uncertainty in Predicted Chemical Properties for the Comparative Toxicity Potentials of Triazoles ".

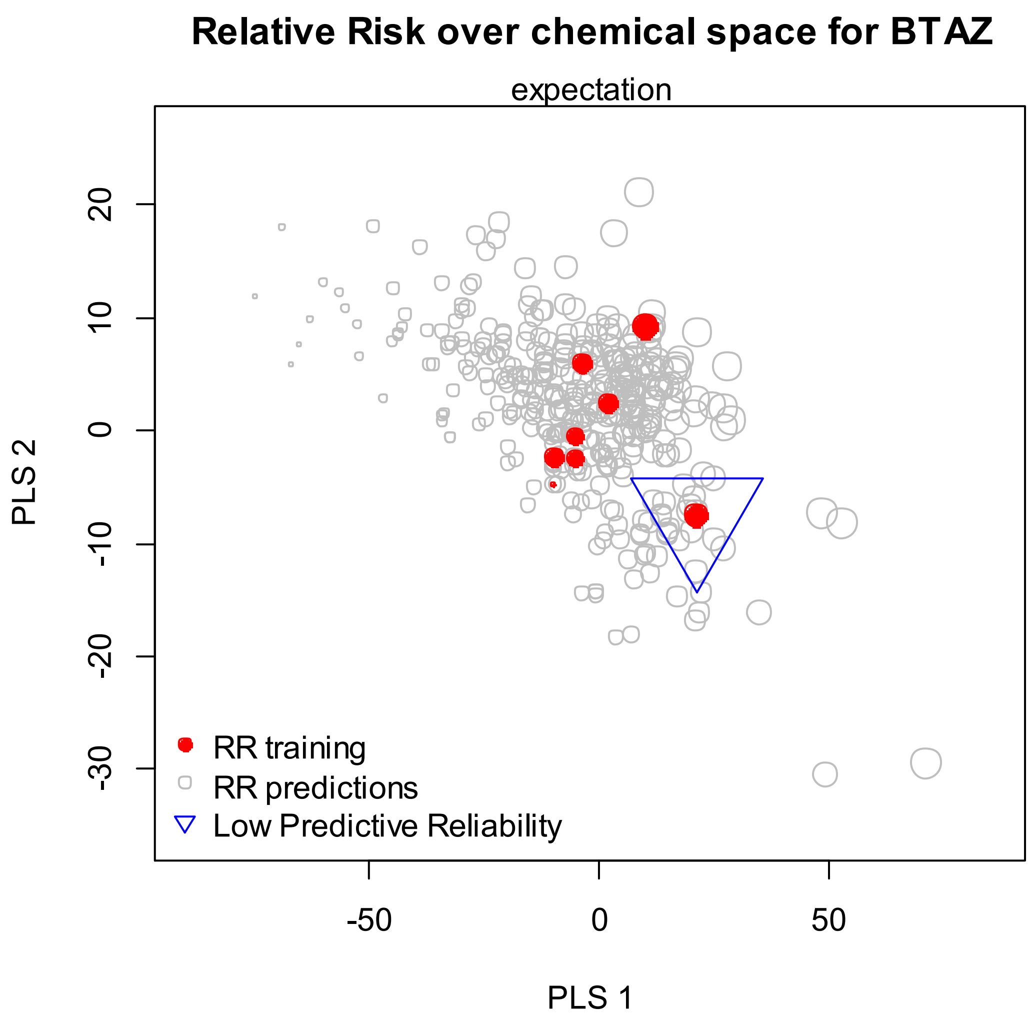


Figure 1. Projections of Relative Risk based on expected values over multivariate characterization of BTAZ molecular space.

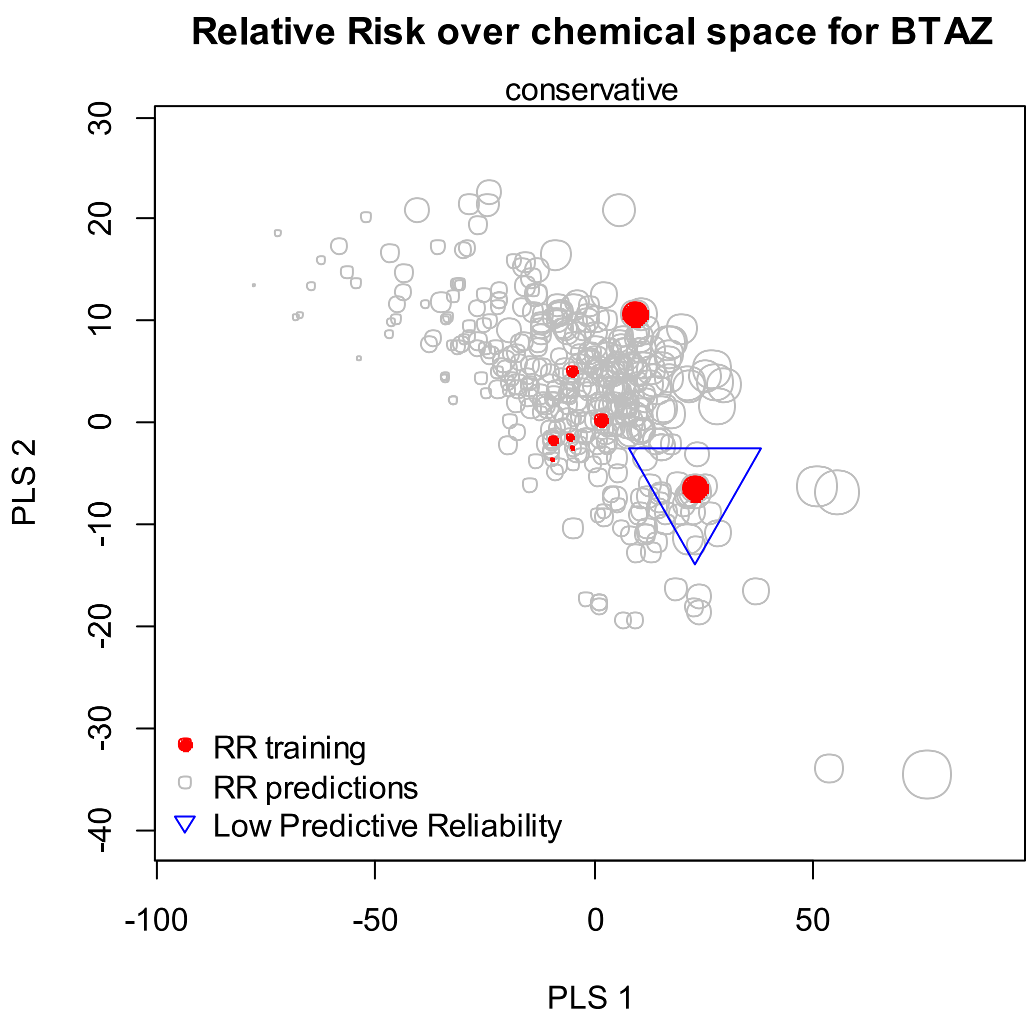


Figure 2. Projections of Relative Risk based on conservative values over multivariate characterization of BTAZ molecular space.

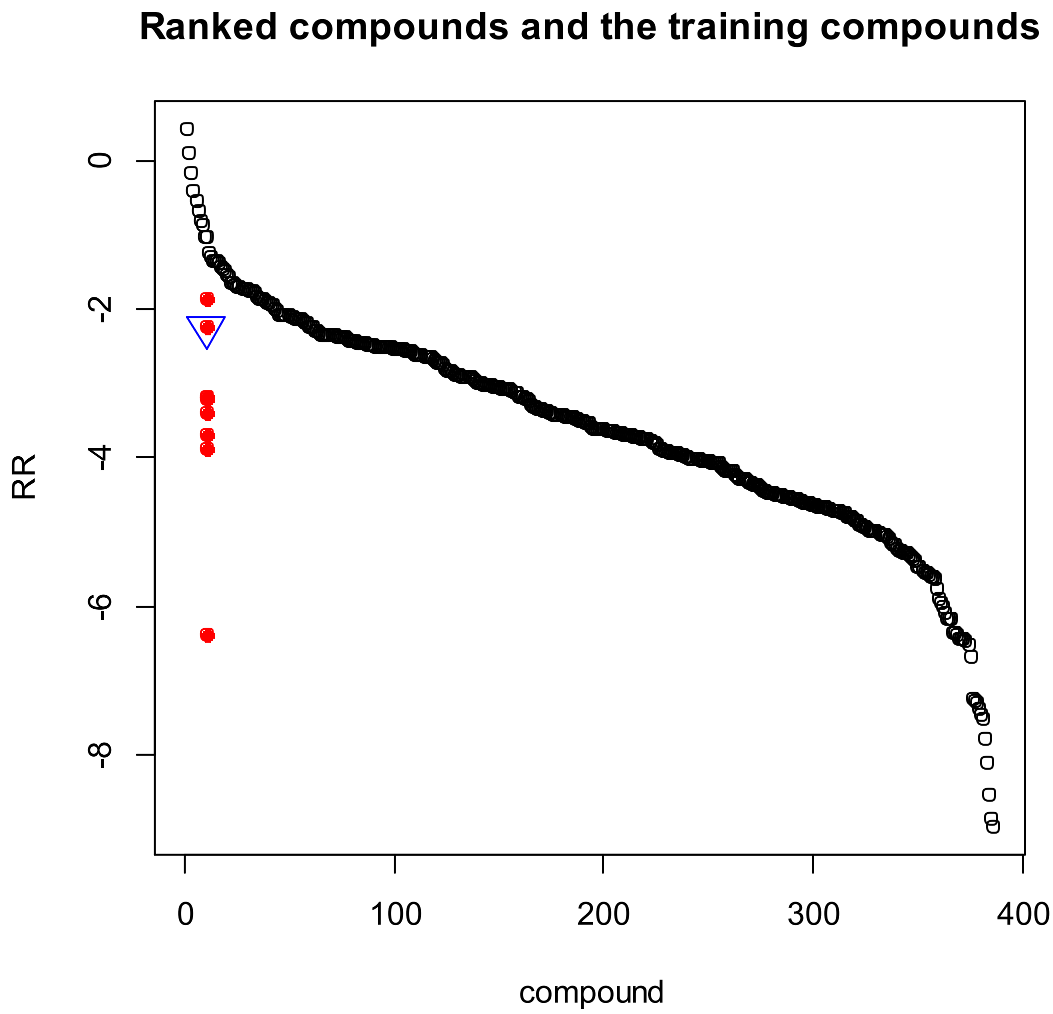


Figure 3. Relative ranking of 386 compounds showing the relative position of the eight compounds that have undergone a full assessment.

Table 1. Uncertainty analysis of Maximum Permissible Emission (MPE) assessed for eight BTAZs. The extended analysis is made to evaluate the influence of non-reliable QSAR predictions which are found for compounds with ID 2 and 7.

Original uncertainty analysis:								
Name	Uniconazole	Diniconazole	Diclobutrazol	Triadimenol-A	Ssf 109	Iponazole	Flupoxam	Sulfen
CAS	083657-17-4	083657-24-3	075736-33-3	089482-17-7	129586-32-9	125225-28-7	119126-15-7	12283
ID	1	2	3	4	5	6	7	8
Mean	4719002.42	14710.84	9522.03	4927.83	3083.73	3296.14	351.00	147.41
5%	7.26	4.08	2.76	2.08	1.26	0.93	0.11	0.132
25%	175.59	43.08	28.41	16.23	10.32	9.65	0.71	0.759
50%	2101.35	268.16	175.76	92.33	59.37	59.34	4.08	3.245
75%	29529.89	1839.19	1170.44	571.82	379.46	384.96	28.14	17.374
95%	1714883.19	25696.27	16840.73	8332.66	5332.57	5805.02	491.72	256.10
Extended uncertainty analysis:								
Mean	4719002.42	14710.84	9522.03	4927.83	3083.73	3296.14	43366.94	147.41
5%	7.26	4.08	2.76	2.08	1.26	0.93	<0.001	0.132
25%	175.59	43.08	28.41	16.23	10.32	9.65	0.004	0.760
50%	2101.35	268.16	175.76	92.33	59.37	59.34	0.31	3.245
75%	29529.89	1839.19	1170.44	571.82	379.46	384.96	456.30	17.374
95%	1714883.19	25696.27	16840.73	8332.66	5332.57	5805.02	93192.96	256.10

Table 2. BTAZ prioritization based on relative risk

CASRN	RECORDID in CADASTER database	Relative Risk	Rank
131-43-1	R3418984	0.418	1
63216-86-4	R3419156	0.101	2
1325-58-2	R3419001	-0.166	3
51627-14-6	R3419122	-0.416	4
89786-04-9	R3419227	-0.537	5
348635-87-0	R3419342	-0.693	6
130-34-7	R3418983	-0.807	7
-	R3419345	-0.859	8
219714-96-2	R3419340	-1.024	9

63251-40-1	R3419157	-1.027	10
173980-17-1	R3419337	-1.241	11
147150-35-4	R3419334	-1.299	12
-	R3419364	-1.353	13
317815-83-1	R3419341	-1.363	14
41083-11-8	R3419102	-1.365	15
136426-54-5	R3419304	-1.378	16
422556-08-9	R3419343	-1.444	17
-	R3419347	-1.459	18
57801-94-2	R3419146	-1.492	19
57801-81-7	R3419145	-1.544	20
139528-85-1	R3419308	-1.568	21
122836-35-5	R3419293	-1.642	22
54123-06-7	R3419136	-1.658	23
145701-21-9	R3419332	-1.666	24
98532-70-8	R3419264	-1.688	25
141079-20-1	R3419330	-1.69	26
98532-73-1	R3419267	-1.73	29
98519-02-9	R3419237	-1.762	32
-	R3419352	-1.778	33
34771-66-9	R3419092	-1.845	34
212201-70-2	R3419339	-1.856	35
-	R3419356	-1.87	36
98519-41-6	R3419255	-1.871	37
145026-81-9	R3419331	-1.884	38
54028-85-2	R3419127	-1.91	39
40054-69-1	R3419101	-1.918	40
145701-23-1	R3419333	-1.942	41
128639-02-1	R3419300	-1.943	42
98519-01-8	R3419236	-2.01	43
6994-51-0	R3419048	-2.016	44
98519-00-7	R3419235	-2.077	47
98519-04-1	R3419238	-2.077	48
54028-86-3	R3419128	-2.085	49
68049-83-2	R3419169	-2.096	50
54028-84-1	R3419126	-2.108	51
54028-83-0	R3419125	-2.122	52
98532-67-3	R3419261	-2.131	53
98532-77-5	R3419270	-2.139	54
28911-01-5	R3419082	-2.142	55
16515-58-5	R3419064	-2.146	56
103112-36-3	R3419280	-2.179	57
103597-45-1	R3419281	-2.196	58
3333-62-8	R3419017	-2.228	59
141079-18-7	R3419328	-2.243	60
116255-48-2	R3419290	-2.251	61

66975-54-0	R3419166	-2.301	62
119446-68-3	R3419292	-2.303	63
-	R3419362	-2.319	64
141079-15-4	R3419325	-2.334	65
103112-35-2	R3419279	-2.339	68
141079-17-6	R3419327	-2.34	69
141079-19-8	R3419329	-2.358	70
119126-15-7	R3419291	-2.358	71
54028-90-9	R3419130	-2.363	72
178928-70-6	R3419338	-2.386	75
-	R3419357	-2.386	76
98532-85-5	R3419275	-2.402	77
139158-26-2	R3419307	-2.42	80
97232-75-2	R3419231	-2.421	81
98519-07-4	R3419241	-2.444	82
106325-08-0	R3419284	-2.448	83
98519-29-0	R3419246	-2.459	85
98519-31-4	R3419248	-2.459	85
98532-71-9	R3419265	-2.459	85
98519-05-2	R3419239	-2.471	87
98967-40-9	R3419276	-2.472	88
85634-51-1	R3419222	-2.49	89
141079-16-5	R3419326	-2.491	90
-	R3419350	-2.514	95
60207-93-4	R3419153	-2.519	96
128625-52-5	R3419299	-2.527	99
-	R3419361	-2.536	102
60207-90-1	R3419152	-2.543	103
98532-83-3	R3419274	-2.545	104
-	R3419353	-2.554	105
19794-93-5	R3419069	-2.558	106
98532-69-5	R3419263	-2.569	107
83366-66-9	R3419218	-2.574	108
-	R3419346	-2.577	109
36791-04-5	R3419097	-2.604	110
54028-92-1	R3419132	-2.604	111
54028-89-6	R3419129	-2.616	112
98519-37-0	R3419253	-2.626	113
54028-81-8	R3419124	-2.637	114
-	R3419354	-2.638	115
98519-33-6	R3419250	-2.651	116
63870-37-1	R3419158	-2.655	117
98519-32-5	R3419249	-2.666	118
54028-95-4	R3419135	-2.703	119
28981-97-7	R3419083	-2.719	120
974-29-8	R3418997	-2.725	121

-	R3419358	-2.735	122
98532-74-2	R3419268	-2.756	123
54028-93-2	R3419133	-2.813	124
98532-81-1	R3419272	-2.826	125
139158-25-1	R3419306	-2.829	126
29975-16-4	R3419086	-2.838	127
141079-13-2	R3419323	-2.859	128
5516-20-1	R3419041	-2.871	129
98518-95-7	R3419232	-2.877	130
64082-38-8	R3419160	-2.891	131
98532-75-3	R3419269	-2.903	132
41735-56-2	R3419115	-2.905	133
98532-65-1	R3419259	-2.909	134
19683-09-1	R3419068	-2.916	135
-	R3419351	-2.918	136
59338-93-1	R3419150	-2.948	137
83044-90-0	R3419216	-2.977	138
-	R3419355	-2.98	139
139158-24-0	R3419305	-3	140
141079-08-5	R3419321	-3.002	141
99793-75-6	R3419278	-3.006	142
125116-23-6	R3419294	-3.006	143
60207-31-0	R3419151	-3.009	144
81518-27-6	R3419207	-3.013	145
141079-14-3	R3419324	-3.024	146
125225-28-7	R3419295	-3.034	147
35515-45-8	R3419093	-3.04	148
81518-41-4	R3419213	-3.05	149
147993-59-7	R3419335	-3.057	150
141078-95-7	R3419313	-3.064	151
141079-12-1	R3419322	-3.065	152
141079-07-4	R3419320	-3.066	153
112281-77-3	R3419287	-3.073	154
83044-91-1	R3419217	-3.074	155
64057-50-7	R3419159	-3.094	156
141079-03-0	R3419318	-3.116	157
131983-72-7	R3419303	-3.119	158
129586-32-9	R3419301	-3.18	159
81518-28-7	R3419208	-3.18	160
54028-91-0	R3419131	-3.182	161
83044-89-7	R3419215	-3.203	162
41735-51-7	R3419112	-3.208	163
41834-21-3	R3419118	-3.238	164
78324-76-2	R3419195	-3.278	165
41735-30-2	R3419105	-3.305	166
86386-73-4	R3419223	-3.323	167

113518-46-0	R3419288	-3.338	168
75020-35-8	R3419173	-3.339	169
70321-86-7	R3419172	-3.348	170
41735-50-6	R3419111	-3.356	171
125306-83-4	R3419297	-3.364	172
54028-94-3	R3419134	-3.371	173
41735-38-0	R3419106	-3.389	174
41735-44-8	R3419109	-3.395	175
-	R3419360	-3.41	176
36325-69-6	R3419094	-3.419	177
3683-95-2	R3419027	-3.426	178
86598-92-7	R3419224	-3.432	179
23633-05-8	R3419072	-3.434	180
55425-38-2	R3419140	-3.454	181
31251-03-3	R3419087	-3.455	182
41735-28-8	R3419103	-3.457	183
24054-57-7	R3419075	-3.461	184
27022-50-0	R3419078	-3.461	185
141079-06-3	R3419319	-3.472	186
1600-61-9	R3419005	-3.48	187
127519-17-9	R3419298	-3.495	188
41735-29-9	R3419104	-3.507	189
10187-79-8	R3419053	-3.514	190
41814-78-2	R3419117	-3.518	191
94361-06-5	R3419229	-3.529	192
1468-26-4	R3419004	-3.533	193
94667-47-7	R3419230	-3.576	194
1326-66-5	R3419002	-3.601	195
70292-10-3	R3419171	-3.604	196
81518-31-2	R3419210	-3.611	197
66535-86-2	R3419165	-3.613	198
76674-21-0	R3419176	-3.614	199
141078-93-5	R3419311	-3.623	200
3896-11-05	R3419031	-3.625	201
141078-94-6	R3419312	-3.63	202
41735-55-1	R3419114	-3.635	203
1704-66-1	R3419007	-3.636	204
79983-71-4	R3419200	-3.655	205
81518-37-8	R3419212	-3.655	206
81518-32-3	R3419211	-3.656	207
81518-26-5	R3419206	-3.675	208
141079-01-8	R3419316	-3.68	209
141079-02-9	R3419317	-3.682	210
41735-54-0	R3419113	-3.688	211
5873-30-3	R3419042	-3.695	212
13091-80-0	R3419057	-3.7	213

78218-51-6	R3419182	-3.718	214
23711-34-4	R3419073	-3.724	215
141078-92-4	R3419310	-3.727	216
98518-96-8	R3419233	-3.73	217
85509-19-9	R3419221	-3.738	218
134-58-7	R3418985	-3.739	219
78218-55-0	R3419186	-3.75	220
10187-86-7	R3419055	-3.757	221
41735-42-6	R3419108	-3.762	222
141079-00-7	R3419315	-3.792	223
81518-29-8	R3419209	-3.799	224
41735-57-3	R3419116	-3.831	225
28401-89-0	R3419081	-3.885	226
99793-38-1	R3419277	-3.888	227
103922-48-1	R3419282	-3.897	228
78371-73-0	R3419197	-3.906	229
41735-45-9	R3419110	-3.907	230
7532-52-7	R3419051	-3.925	231
114369-43-6	R3419289	-3.93	232
-	R3419348	-3.932	233
41735-41-5	R3419107	-3.939	234
31701-42-5	R3419089	-3.944	235
36437-37-3	R3419096	-3.956	236
10187-84-5	R3419054	-3.969	237
27210-18-0	R3419079	-3.971	238
3864-99-1	R3419030	-3.975	239
78371-74-1	R3419198	-4	240
75736-33-3	R3419174	-4.001	241
66104-34-5	R3419161	-4.011	242
78150-00-2	R3419180	-4.015	243
56383-06-3	R3419141	-4.015	244
78149-96-9	R3419179	-4.024	245
78218-58-3	R3419189	-4.033	246
25973-55-1	R3419076	-4.035	247
10187-89-0	R3419056	-4.036	248
56881-36-8	R3419144	-4.037	249
66104-44-7	R3419162	-4.045	250
141078-91-3	R3419309	-4.06	251
59338-92-0	R3419149	-4.066	252
2683-90-1	R3419011	-4.069	253
149508-90-7	R3419336	-4.07	254
56383-11-0	R3419142	-4.08	255
78371-72-9	R3419196	-4.109	256
141078-99-1	R3419314	-4.155	257
1031-47-6	R3418999	-4.169	258
43029-44-3	R3419120	-4.172	259

83657-24-3	R3419220	-4.172	260
59026-08-3	R3419147	-4.181	261
78218-56-1	R3419187	-4.184	262
56396-43-1	R3419143	-4.232	263
15805-10-4	R3419063	-4.265	264
88671-89-0	R3419225	-4.281	265
-	R3419349	-4.289	266
3147-75-9	R3419013	-4.294	267
3652-27-5	R3419023	-4.308	268
66246-88-6	R3419163	-4.329	269
2440-22-4	R3419009	-4.34	270
59338-86-2	R3419148	-4.354	271
32723-50-5	R3419091	-4.367	272
5472-71-9	R3419040	-4.373	273
55179-31-2	R3419137	-4.388	274
3652-31-1	R3419024	-4.425	275
36411-52-6	R3419095	-4.44	276
78218-53-8	R3419184	-4.446	277
6299-39-4	R3419045	-4.466	278
1028-08-6	R3418998	-4.474	279
24017-47-8	R3419074	-4.475	280
37160-06-8	R3419098	-4.478	281
112143-82-5	R3419286	-4.487	282
55375-40-1	R3419139	-4.489	283
18811-70-6	R3419067	-4.494	284
80584-89-0	R3419203	-4.507	285
80584-88-9	R3419202	-4.518	286
78150-02-4	R3419181	-4.527	287
31409-18-4	R3419088	-4.528	288
78592-90-2	R3419199	-4.546	289
60932-58-3	R3419154	-4.555	290
6054-53-1	R3419043	-4.556	291
39968-33-7	R3419100	-4.56	292
78218-59-4	R3419190	-4.566	293
3846-71-7	R3419029	-4.587	294
55219-65-3	R3419138	-4.592	296
82200-72-4	R3419214	-4.592	296
89482-17-7	R3419226	-4.592	296
107534-96-3	R3419285	-4.595	298
947-85-3	R3418996	-4.625	299
3147-76-0	R3419014	-4.639	300
66492-64-6	R3419164	-4.641	301
2338-12-07	R3419008	-4.654	302
32362-89-3	R3419090	-4.655	303
78218-54-9	R3419185	-4.656	304
76738-62-0	R3419177	-4.663	305

43121-43-3	R3419121	-4.665	306
67465-03-6	R3419167	-4.678	307
77314-77-3	R3419178	-4.688	308
125304-04-3	R3419296	-4.712	309
1123-54-2	R3419000	-4.714	310
42509-80-8	R3419119	-4.717	311
104958-85-2	R3419283	-4.717	312
-	R3419365	-4.728	313
78218-61-8	R3419192	-4.738	314
78218-52-7	R3419183	-4.749	315
865318-97-4	R3419344	-4.786	316
3652-32-2	R3419025	-4.801	317
61691-97-2	R3419155	-4.802	318
78218-60-7	R3419191	-4.823	319
129909-90-6	R3419302	-4.837	320
83657-17-4	R3419219	-4.846	321
78218-65-2	R3419193	-4.906	322
944-91-2	R3418995	-4.912	323
938-56-7	R3418992	-4.925	324
94-97-3	R3418981	-4.928	325
78218-57-2	R3419188	-4.949	326
3652-25-3	R3419022	-4.979	327
21050-95-3	R3419070	-4.989	330
3142-42-5	R3419012	-5.002	331
80595-74-0	R3419205	-5.036	332
2592-95-2	R3419010	-5.039	333
15421-84-8	R3419061	-5.041	334
939-08-2	R3418994	-5.073	335
3652-23-1	R3419021	-5.089	336
38942-51-7	R3419099	-5.153	337
273-40-5	R3418987	-5.16	338
3652-22-0	R3419020	-5.183	339
3770-47-6	R3419028	-5.23	340
18076-61-4	R3419066	-5.249	341
76608-88-3	R3419175	-5.251	342
80301-64-0	R3419201	-5.27	343
932-64-9	R3418991	-5.276	344
78218-66-3	R3419194	-5.287	345
27799-91-3	R3419080	-5.31	346
29440-31-1	R3419084	-5.34	347
5369-84-6	R3419039	-5.348	348
69141-50-0	R3419170	-5.389	349
16584-05-7	R3419065	-5.453	350
939-07-1	R3418993	-5.463	351
67465-05-8	R3419168	-5.531	352
136-85-6	R3418986	-5.533	353

15497-45-7	R3419062	-5.545	354
3663-24-9	R3419026	-5.568	355
29878-31-7	R3419085	-5.601	356
10109-05-4	R3419052	-5.611	357
13351-73-0	R3419059	-5.621	358
3310-68-7	R3419016	-5.761	359
95-14-7	R3418982	-5.892	360
14803-99-7	R3419060	-5.942	361
7411-23-6	R3419050	-6.003	362
4343-73-1	R3419034	-6.094	363
4928-87-4	R3419036	-6.164	364
5302-27-2	R3419038	-6.167	365
26621-45-4	R3419077	-6.174	366
4314-22-1	R3419033	-6.344	367
3357-42-4	R3419018	-6.352	368
1680-44-0	R3419006	-6.377	369
6085-94-5	R3419044	-6.423	370
4368-68-7	R3419035	-6.425	371
4184-79-6	R3419032	-6.442	372
21532-04-7	R3419071	-6.472	373
4928-88-5	R3419037	-6.524	374
6789-99-7	R3419046	-6.678	375
3641-10-09	R3419019	-7.243	376
3232-84-6	R3419015	-7.265	377
1455-77-2	R3419003	-7.291	378
53817-16-6	R3419123	-7.374	379
13257-88-0	R3419058	-7.455	380
6818-99-1	R3419047	-7.501	381
288-88-0	R3418989	-7.765	382
61-82-5	R3418980	-8.091	383
7170-01-06	R3419049	-8.531	384
584-13-4	R3418990	-8.864	385
288-36-8	R3418988	-8.95	386
98519-35-8	R3419252	-2.534	100.5
98532-82-2	R3419273	-2.534	100.5
98519-25-6	R3419243	-1.718	27.5
98532-66-2	R3419260	-1.718	27.5
98519-26-7	R3419244	-1.756	30.5
98532-68-4	R3419262	-1.756	30.5
80584-90-3	R3419204	-4.985	328.5
94270-86-7	R3419228	-4.985	328.5
98519-06-3	R3419240	-2.067	45.5
98519-43-8	R3419256	-2.067	45.5
98519-30-3	R3419247	-2.339	66.5
98532-72-0	R3419266	-2.339	66.5
98519-28-9	R3419245	-2.377	73.5

98532-64-0	R3419258	-2.377	73.5
98519-34-7	R3419251	-2.418	78.5
98532-80-0	R3419271	-2.418	78.5
98518-99-1	R3419234	-2.499	91.5
98519-39-2	R3419254	-2.499	91.5
-	R3419359	-2.502	93.5
-	R3419363	-2.502	93.5
98519-24-5	R3419242	-2.52	97.5
98519-49-4	R3419257	-2.52	97.5

Appendix 6

Case study 6: The impact of uncertainty in QSAR integrated hazard assessment

A QSAR integrated hazard assessment was built for (Benzo)Triazoles (BTAZs) to illustrate QSAR integrated Chemical Safety Assessment as performed in the CADASTER project. A compound was classified as potentially toxic by comparing a derived hazardous concentration in aquatic environment to a predefined threshold. An assessment was based on QSAR predictions of aquatic toxicity on three species:, algae, daphnia and fish. The QSARs are described in [1]. First we identified the lowest Effect Concentration where 50% of individuals of the most sensitive population (among those tested) are affected, i.e. EC50. Following the recommendations by REACH [2], we classified a compound as "very toxic" to the aquatic environment if the EC50 value on the most sensitive (evaluated) species, min{EC50}, was smaller than 1 mg/L.

QSAR predictions were derived for 386 BTAZs based on consensus modelling. With the aim to illustrate the effect of considering uncertainty in QSAR predictions, we based hazard assessments on either QSAR prediction without uncertainty (i.e. point predictions) or with uncertainty (i.e. by a probability distribution for the error). The underlying QSARs predicted point predictions only. Uncertainty in QSAR predictions was characterized as a Normal distribution (symmetric bell shaped curve) with the point prediction as its mean and predictive error as its standard deviation. Predictive error was assigned the Root Mean Squared Error (RMSE) value derived for the training QSAR data sets. The RMSE was chosen as a good approximation of the average predictive error for compounds that are in the models domain of applicability. This approach to assess the predictive distribution in a QSAR prediction may be categorized as an expert judgment informed by statistical measures. All compounds were predicted by the three QSARs even though some of them fell out of one or more applicability domains. Compounds with the least reliable predictions were identified by the Maximum Absolute Difference (MAD) between individual predictions in the consensus modelling. Compounds with for which at least one MAD score among the three QSAR predictions were larger than 0.9 were judged to be given less reliable assessment.

The hazard assessment based on QSAR predictions with uncertainty were done by Monte Carlo simulation, where random samples were derived from the corresponding predictive distributions and, in each iteration, the min{EC50} value stored. The resulting uncertainty in aquatic toxicity (i.e. min{EC50} values) is described by a probability distribution. From this probability distribution we calculated the expected value, the median and the 5^{th} percentile. A best guess or most likely value of aquatic toxicity can be provided by the median and expected value. The median does not consider extreme values, while the expected value weights all possible values with their likelihood of occurring. Differences in expected and median values are found for skewed distributions with the presence of either high or low extreme events. When the interspecies variability in sensitivity is relatively larger than the uncertainty in individual QSAR predictions, the uncertainty in the min{EC50} value will be dominated by the uncertainty in the most sensitive species. In the QSAR models provided here these predictions have a symmetric distribution. When species interspecies variability is small in comparison to QSAR uncertainty, the minimum out of three values should result a skewed distribution. In this case-study the differences between median and expected values were negligible, meaning that the uncertainty in the classification variable was rather symmetric.

Thus, uncertainty in the output of the hazard assessment was considered in four ways, without considering QSAR uncertainty which gave only one value on the min{EC50}, the expected, median or 5^{th} percentile of min{EC50} derived from considering uncertainty in QSAR predictions. A list of 385 BTAZs were classified as potentially toxic or not based on the four different ways to consider uncertainty. We calculated the number of compound for which the consideration of uncertainty resulted in different classifications (Figure A1.1). In particular we were interested in the number of compounds for which the toxicity classification changed from not toxic to potentially toxic when considering uncertainty in QSAR predictions or when taking a more risk adverse attitude (Figure A1.2).

Consideration of QSAR uncertainty resulted in more cautious classifications (Figure A1.2) and an avoidance of making errors of type II. 19 out of 386 compounds were classified as toxic after QSAR uncertainty in input had been taken into account. Adding risk averse behaviour, an additional amount of 115 compounds were classified as potentially toxic.

We found that using conservative values for QSAR predictions (5^{th} percentile) as input to the hazard assessment resulted in an increased probability of making error of type II compared to classifications based on the 5^{th} percentile of the output (Figure A1.1 d).

To conclude, the impact on decision making from considering uncertainty in QSAR predictions was a reduced probability of making errors of type II. This effect was found when the full predictive distribution was used as input. Reducing the information on predictive uncertainty to a conservative value in the input to the hazard assessment did not automatically lead to more conservative classifications. Seven compounds were classified as non-toxic under the conservative assessment, which were classified as potentially toxic based on hazard assessment with probabilistic uncertainty analysis and risk adverse behaviour. Using conservative values to specify input both increase the probability of committing errors of type II, hinder the decision maker to be risk neutral, and force to decision maker to be risk averse to an unknown degree.

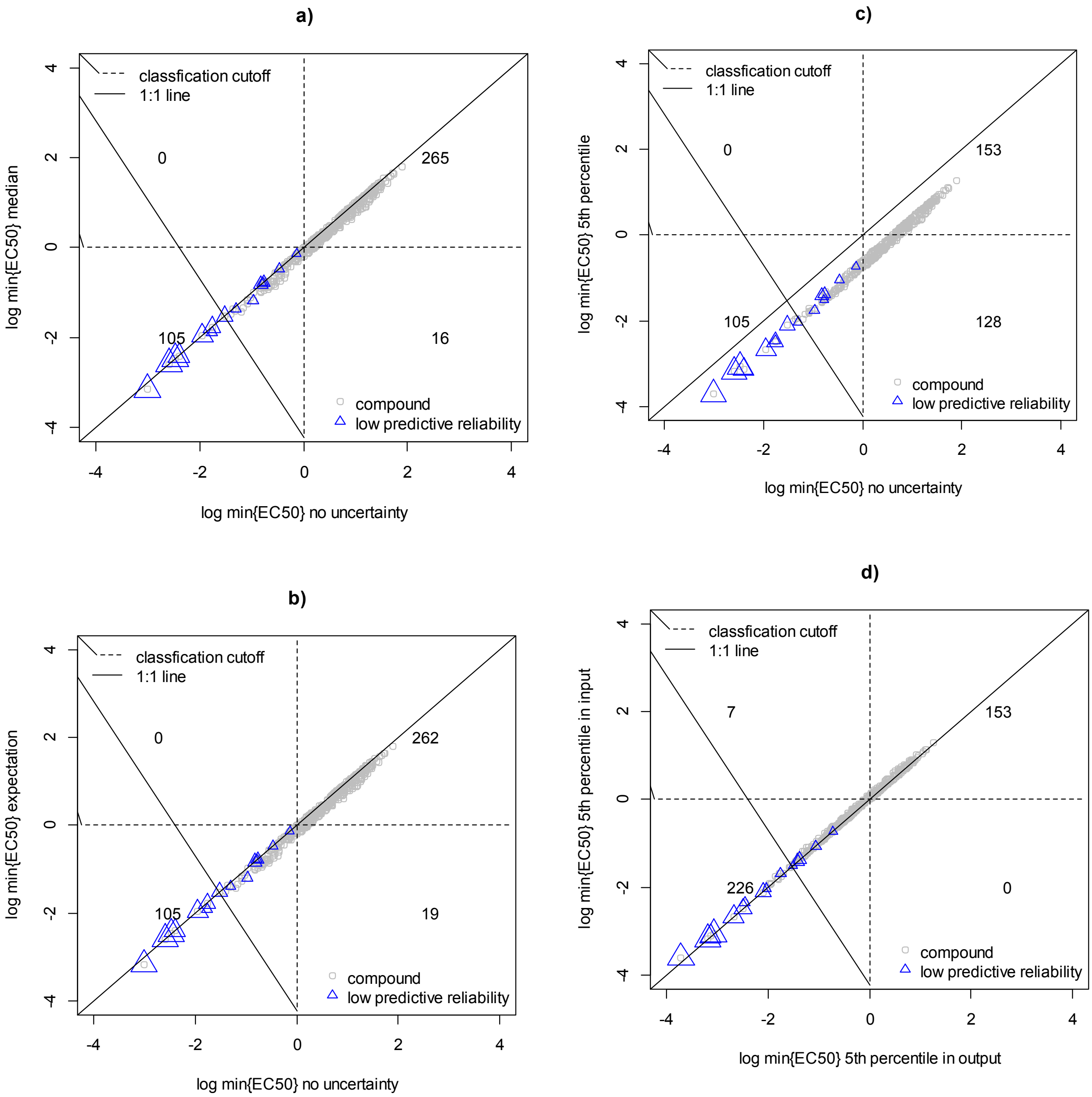

a)
classfication cutoff
1:1 line
0
265
105
16
compound
low predictive reliability
log min{EC50} median
log min{EC50} no uncertainty
b)
classfication cutoff
1:1 line
0
262
105
19
compound
low predictive reliability
log min{EC50} expectation
log min{EC50} no uncertainty
c)
classfication cutoff
1:1 line
0
153
105
128
compound
low predictive reliability
log min{EC50} 5th percentile
log min{EC50} no uncertainty
d)
classfication cutoff
1:1 line
7
153
226
0
compound
low predictive reliability
log min{EC50} 5th percentile in input
log min{EC50} 5th percentile in output

Figure A1.1. Comparison of aquatic toxicity for 385 BTAZs derived by QSAR integrated hazard assessment under different ways to consider uncertainty in QSAR predictions and attitudes to uncertainty in the assessment output. Compounds with less reliable predictions were identified by the maximum absolute difference between individual predictions in QSAR consensus modelling.

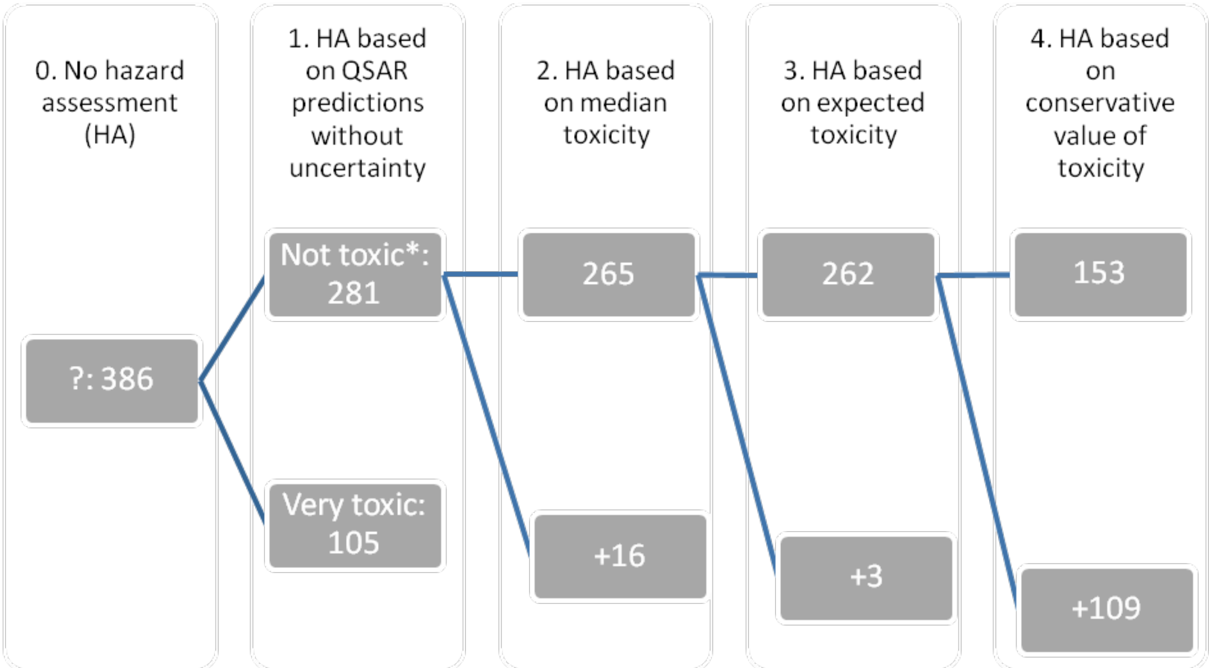


Figure A1.2. The number of BTAZs compounds classified as very toxic or not (including potentially*) toxic under the different treatments of QSAR uncertainty both in input and in the output of the assessment. Uncertainty in QSAR predictions is considered in alternatives 2 to 4.

References

1. Cassini, S., Kovarich, S., Papa, E., et al. (in review). Evaluation of CADASTER QSAR models for aquatic toxicity of (benzo-)triazoles and prioritization by consensus.

2. European Commission (EC). (1991). Directive 67/548/EEC, 1991 (Annex VI). General Classification and Labelling Requirements for Dangerous Substances and Preparations.

Appendix 7

Case study 7: Uncertainty analysis in QSAR integrated risk assessment

A non-safe emission occurs when the Environmental Concentration (EC), exceeds the No Effect Concentration (NEC). Fate and effect assessments were made to find the Predicted EC (PEC) and Predicted NEC (PNEC) of eight BTAZs (Table A2.1). Uncertainty in the value of these quantities was expressed as probability distributions around the ratio between PNEC and PEC. When there is strong evidence that EC will be larger than NEC the decision is to apply risk management to reduce emissions, or refine the assessment to improve the evidence. We refer to these actions as “to regulate”. The case-study used several QSARs to assess PEC and PNEC for risk assessment. The purpose of the cases-study was to demonstrate the integration of QSARs into probabilistic risk assessment and was therefore based on a hypothetical common emission scenario for all evaluated compounds and did not consider all relevant non-QSAR sources of uncertainty.

The case-study was based on the QSAR integrated risk assessment described in [1]. Fate assessments (PEC) were made using Simplebox under a unit emission rate. Effect assessments derived PNEC as the minimum out of QSAR predicted EC50 values on three aquatic species divided by an assessment factor of 1000. The level of emission was here adjusted to 500 kg/day in order to obtain compounds classified as safe and as risk.

Assessment was done on two levels of complexity in the consideration of uncertainty. The output from a deterministic risk assessment (tier 0), i.e. where uncertainty is not considered, results in most likely values on PEC and PNEC. Here all eight compounds had a Risk Characterization Ratio (RCR) below one which would indicate that they are safe (Figure A2.1). Seeking to avoid making erroneous risk classifications probabilistic assessments was performed to uncertainty (tier 3). The probabilistic evaluation of risk showed that compound were still safe since the probability of PEC exceeding PNEC was less than 5% (Figure A2.1). Considering sources of uncertainty does in general lead to safer decisions, and in that respect is uncertainty from QSAR predictions no exception.

Two of the compounds (ID 2 and 7) were judged as being on the borderline of at least one QSAR model used as input to the assessment (see [1]). To evaluate the influence of these QSAR predictions with lower confidence we made a reassessment of risk where the corresponding predictive distributions had been enlarged by an arbitrary factor (here the standard deviation in the predictive distribution had been multiplied by 10). This resulted in an enlarged risk (p+ in Figure A2.1). This sensitivity analysis showed that risk classification of compound ID 7 was sensitive to the lower confidence from QSAR predictions, as the 95th percentile of the RCR changed from being less than, to larger than one. In this case, the assessment of compound ID 8 may need to use other sources of background information to hold the same quality as the others, since we still may be unsure whether the assessment was acceptable or not.

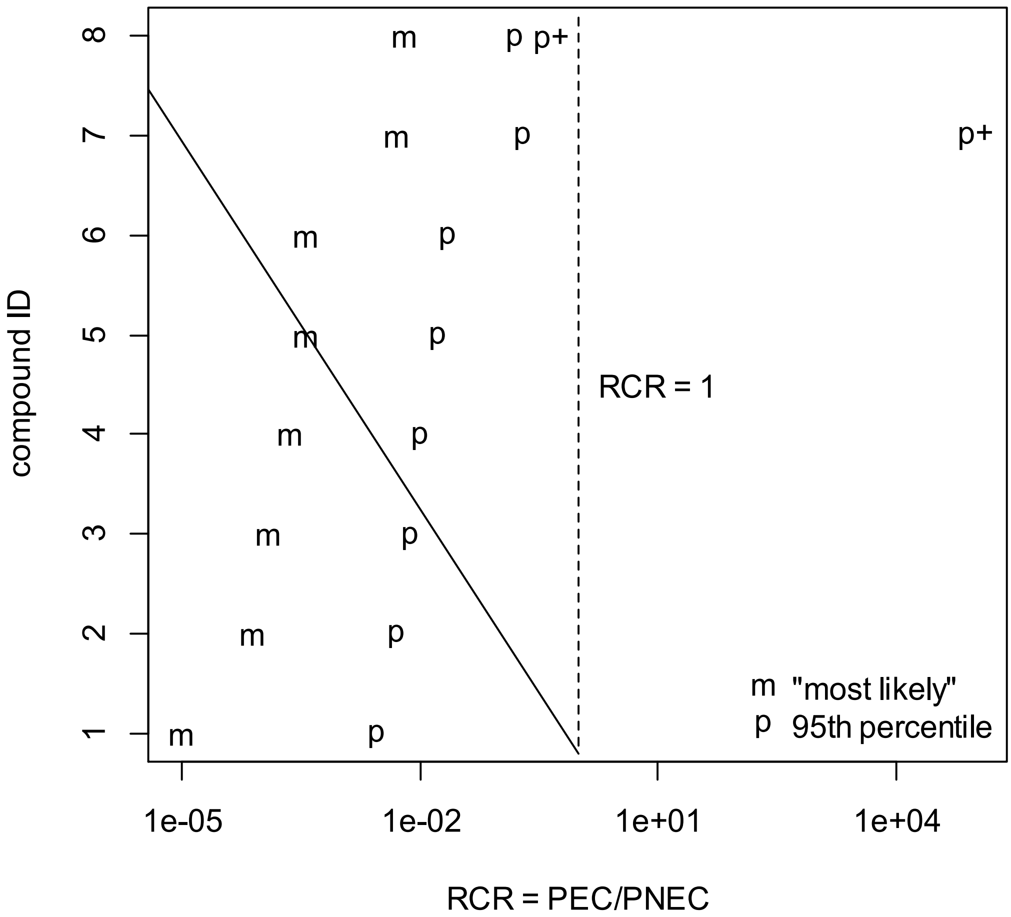


Figure A2.1. Risk Characterization Ratio under deterministic (m) and probabilistic (p) risk assessments of eight BTAZs showing the influence by considering uncertainty in input parameters. A reassessment using enlarged uncertainty in unreliable QSAR predictions (p+) were made for compound 7 and 8. Compound IDs are the same as in Table A2.1.

Table A2.1. Uncertainty analysis of Risk Characterization Ratio (RCR) given a common emission scenario based on assessments of eight BTAZ's. The extended analysis is made to evaluate the influence of non-reliable QSAR predictions which are found for compound with ID 2 and 7.

Original uncertainty analysis:								
Name	Uniconazole	Diniconazole	Diclobutrazol	Triadimenol-A	Ssf 109	Iponazole	Flupoxam	Sulfentrazone
CAS	083657-17-4	083657-24-3	075736-33-3	089482-17-7	129586-32-9	125225-28-7	119126-15-7	122836-35-5
ID	1	2	3	4	5	6	7	8
Mean	-6.67	-4.17	-3.98	-3.69	-3.49	-3.52	-2.55	-2.17
5%	-0.86	-0.61	-0.44	-0.32	-0.10	0.03	0.97	0.88
25%	-2.24	-1.63	-1.45	-1.21	-1.01	-0.98	0.15	0.12
50%	-3.32	-2.43	-2.24	-1.97	-1.77	-1.77	-0.61	-0.51
75%	-4.47	-3.26	-3.07	-2.76	-2.58	-2.59	-1.45	-1.24
95%	-6.23	-4.41	-4.23	-3.92	-3.73	-3.76	-2.69	-2.41
Extended uncertainty analysis:								
Mean	-6.67	-4.17	-3.98	-3.69	-3.49	-3.52	-4.64	-2.17
5%	-0.86	-0.61	-0.44	-0.32	-0.10	0.03	8.66	0.88
25%	-2.24	-1.63	-1.45	-1.21	-1.01	-0.98	2.39	0.12
50%	-3.32	-2.43	-2.24	-1.97	-1.77	-1.77	0.51	-0.51
75%	-4.47	-3.26	-3.07	-2.76	-2.58	-2.59	-2.66	-1.24
95%	-6.23	-4.41	-4.23	-3.92	-3.73	-3.76	-4.97	-2.41

Reference: Golsteijn, L., Iqbal, M. S., Cassani, S., et al. (in review). The Relative Importance of Uncertainty in Predicted Chemical Properties for the Comparative Toxicity Potentials of Triazoles.

Appendix 8

Case study 8: Prioritization based on hazard assessment of PFCs

PNEC assessment

PNEC values can be estimated by extrapolation from species toxicity data using the method of assessment factor (AF) or method of Species Sensitivity Distribution (SSD) (B.7.2. ECHA 2008). The AF method is to identify the appropriate assessment factor as a function of available information and divide the lowest effect concentration with the assessment factor (Table 1).

The SSD method uses data on long-term tests and requires, at minimum, 10 species to be valid (R.10.3.1.3 ECHA 2012). Here we demonstrate the use of QSAR integrated SSD even though we do not have sufficient species information to support this approach. The SSD method is to fit a Species Sensitivity Distribution to log EC50 values and derive the hazardous concentration considering uncertainty from sample size (Aldenberg and Jaworska 2000). Uncertainty in the hazardous concentration based on EC50 values as point predictions are derived as the non-central Student-t distribution. The PNEC value is then the median of the distribution for the Hazardous Concentration (HC). Uncertainty in QSAR predictions were considered by performing an outer loop using Monte Carlo simulation to sample from the predictive distributions and storing the median HC value in each iteration. PNEC was derived by dividing HC with a factor of 1000 to open up for a comparison to the AF method. A point value of the PNEC was taken as the median (Alt C) or the 5th percentile (Alt D) of the distribution for the Hazardous Concentration.

First we conclude that when ranking without considering quality of available information, there is no need to derive the AF since we only make relative comparisons and the AF is equal for all compounds. The need to consider AF is introduced when the PNEC is derived for the purpose of risk assessment to be compared to a threshold for classification or another quantity such as the Predicted Environmental Concentration.

Results from long-term tests are usually expressed as EC10/NOEC for a sublethal parameter and preferred to those of short-termed tests, expressed as EC50 or LC50 values (B.7.2.1 ECHA 2008). Since the hazard assessment was based on QSARs that predict short-term (or acute) aquatic toxicity, the accepted procedure to arrive at a PNEC was to divide the lowest EC50 value an assessment factor of 1000. This assessment factor is made up of a general acute-to-chronic ratio of 100 plus a safety factor of 10 to account for the fact that only 4 species are sampled.

QSAR predictions

Hazard assessment of PFCs where limited to PNEC for freshwater based on experimental data on a few PFCs that had been derived within the project. QSARs developed for PFCs using the relationship between the fluorinated carbon length (nC) and log EC50 values for four species were used to predict aquatic toxicity (Ding, Wouterse et al. 2012; Ding, Fromel et al. 2012). The QSAR training data contained 7 PFCs selected for the toxicity assessment on the basis of their chemical structural resemblance. The selected PFCs were (1) perfluorobutanoic acid (PFBA; CAS no. 375-22-4, purity 98%), (2) 2,2,3,3,4,4,5,5-Octafluoro-1-pentanol (5H 4:1 FTOH; CAS no. 355-80-6, purity 98%), (3) PFOA (CAS no. 335-67-1, purity 96%), (4) perfluorononanoic acid (PFNA; CAS no. 375-95-1, purity 97%), (5) perfluorodecanoic acid (PFDA; CAS no. 335-76-2, purity 98%), (6) perfluoroundecanoic acid (PFUnA; CAS no. 2058-94-8, purity 95%), and (7) PFDoA (CAS no. 307-55-1, purity 97%). Experimental toxicity tests on four species for all these seven compounds constituted the training data for the QSAR, where descriptors were carbon chains of length 3, 4, 7, 8, 9, 10, and 11.

This is a typical small QSAR data set that poses challenges to quantitatively assess the QSAR and quantitatively assess the uncertainty in its predictions. For example, there had been no external validation. Despite the small data size, may predictions be supported due to the clear mechanistic understanding associated with these QSARs. The QSARs in the original papers had been fitted by Ordinary Least Squares regression and provided predictions without uncertainty. We suggest that uncertainty in QSAR predictions is easier to implement in risk assessment when framed in a Bayesian framework, but since this is not the prevailing statistical framework for OLS models, we build a corresponding and similar Bayesian model. QSAR predictions with uncertainty were here modelled in a Bayesian framework using Bayesian regularized regression with lasso penalty (Li, Xi et al. 2010). The difference in predictions from OLS and the mean predictive posterior from the Bayesian model were negligible. The predictive distribution over the range of nC are shown in Figure 1a for log EC50 48 h for *C. sphaericus*. Predictive reliability was evaluated by considering the range of descriptor values and by comparing hat values, which is the diagonal of the information matrix, to a cut off of 3 times the average hat value (Figure 1b).

Modelled response

The effective concentrations had been evaluated on tests on root elongation of lettuce (L. sativa) seeds, photosynthesis of green algae (P. subcapitata), inhibition effects on D. magna and C. sphaericus. The QSAR predict logEC50 in units of mM = 10 -3 mol/L. This was transformed to mg/L by multiplying by the molecular weight given in g/mol.

Derivation of Relative Hazard – degree of conservative assumptions

A point prediction from a QSAR that predict log EC50 is the mean in the predictive distribution, which coincide with the median when the distribution for the error is symmetric. The transformed predictive distribution when going from log EC50 to EC50 is skewed and its mean do not correspond to its median. A point prediction is the median value, which remains a median through any monotonous (i.e. either increasing or decreasing) transformations. It preserves values at the central domain of a distribution, but fails to include the influence of extreme values.

We could either sample from the predictive distributions, and derive the distribution of min values and take the 5^{th} percentile of this distribution (Alt A), or select conservative estimates such as 5^{th} percentile from every predictive distribution and take the minimum of those (Alt B). Comparison in Figure 2 shows that alternative A in this case generates more conservative min EC 50 values. PNEC = 10 ^ (min{log EC water}) / 1000.

PNEC values derived from the AF method and using SSD were compared to get information about different treatments of uncertainty in QSAR predictions and species variability. Hazard assessment was in Alternative C based on the 5^{th} percentile in fitted SSD based on conservative QSAR predictions,. The fourth alternative (D) was to base the hazard assessment on the 5^{th} percentile in fitted SSD based on double loop sampling from underlying predictive distributions of the QSAR predictions. Alternative D generated most conservative values on PNEC (Figure 3) and was chosen for the ranking. Without the SSD, alternative A was most conservative, which points out that it may be worthwhile to consider QSAR predictions with uncertainty as the predictive distribution when input, instead of a point value before an assessment.

The size of the circles grows as the PNEC value becomes smaller (Figure 4), i.e. when the compound is regarded as more toxic to the environment (water).

Predictive reliability

Now we ask for which of these assessment the underlying QSARs on aquatic toxicity, based on number of carbon atoms, are applicable. Chemical domain has been identified to be PFCs excluding those that are an acid or alcohol.

According to the cut-off value 3 times the average hat value for the training data set, are predictions of PFCs with a nC with two or less, or 13 or more, not reliable (Figure 5). The communication of confidence in predictions can be facilitated by intelligent graphics (Figure 6).

Considering relative lower confidence show that PFCs with 13 or more nC receive the lowest HC value before changing unit from mmol/L to mg/L, and are potentially more hazardous (Figure 7).

Ranking

The list of 93 PFCs were ranked according to the relative hazard calculated as –log PNEC and the most conservative way to assess min (EC50). The confidence in assessments that can be understood from the statistical model was dependent on the length of the number of carbon atoms. Since the relative hazard was based on min(EC50) and molecular weight, the lower predictive reliability appeared on difference places in the ranked list (Figure 8). The compounds identified as of most concern are assessed with high confidence. Compounds on the lower part of the list and with low confidence may not be acceptable assessments. The list of ranked compounds is provided in Table 2.

References

Aldenberg, T. and J. S. Jaworska (2000). "Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions." Ecotoxicology and Environmental Safety 46(1): 1-18.

Ding, G., M. Wouterse, et al. (2012). "Toxicity of polyfluorinated and perfluorinated compounds to lettuce (Lactuca sativa) and green algae (Pseudokirchneriella subcapitata)." Arch Environ Contam Toxicol 62(1): 49-55.

Ding, G. H., T. Fromel, et al. (2012). "Acute toxicity of poly- and perfluorinated compounds to two cladocerans, Daphnia magna and Chydorus sphaericus." Environ Toxicol Chem 31(3): 605-610.

ECHA (2008). Guidance on information requirements and chemical safety assessment, European Chemicals Agency Helsinki Finland.

ECHA (2012). R.10: Characterisation of dose [concentration]-response for environment, Guidance on information requirements and chemical safety assessment, European Chemicals Agency Helsinki Finland.

Li, Q., R. Xi, et al. (2010). "Bayesian Regularized Quantile Regression." Bayesian Analysis 5(3): 533-556.

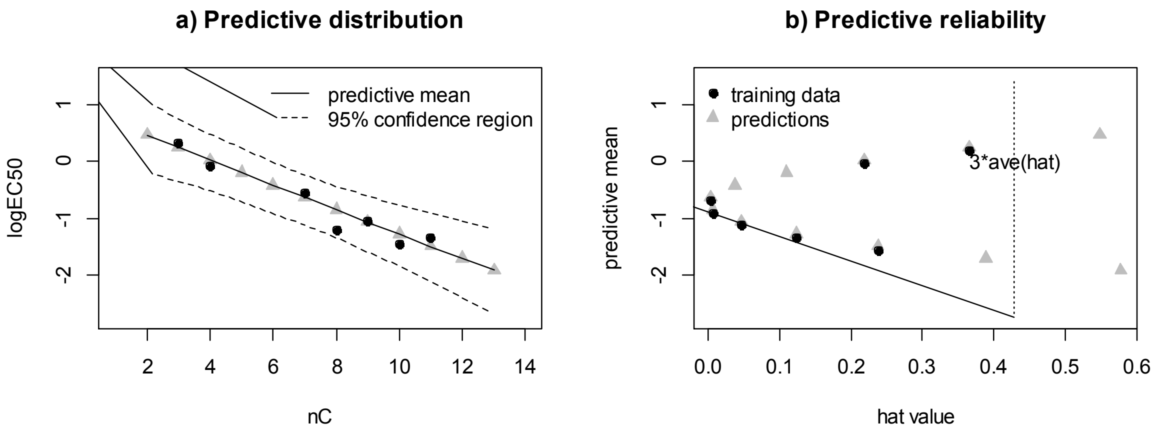


Figure 1. Quantitative and qualitative uncertainty in QSAR predictions.

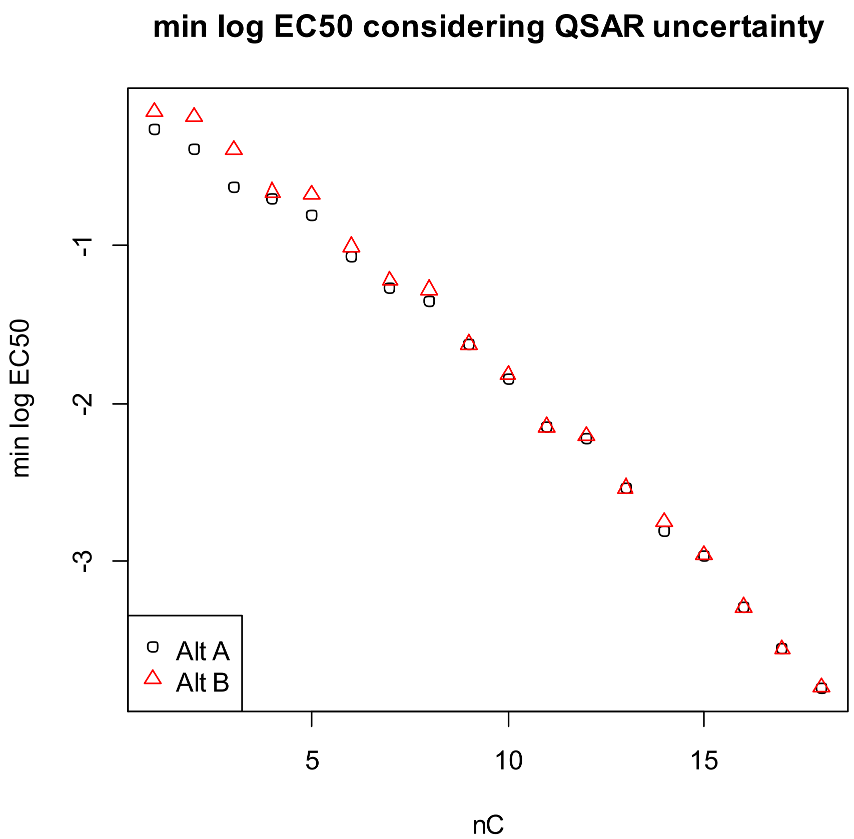


Figure 2. Aquatic toxicity comparing two ways to do the AF method given uncertainty in QSAR predictions, as conservative values in the input (Alt A) or full characterization of uncertainty in input and a conservative value of the output (Alt B).

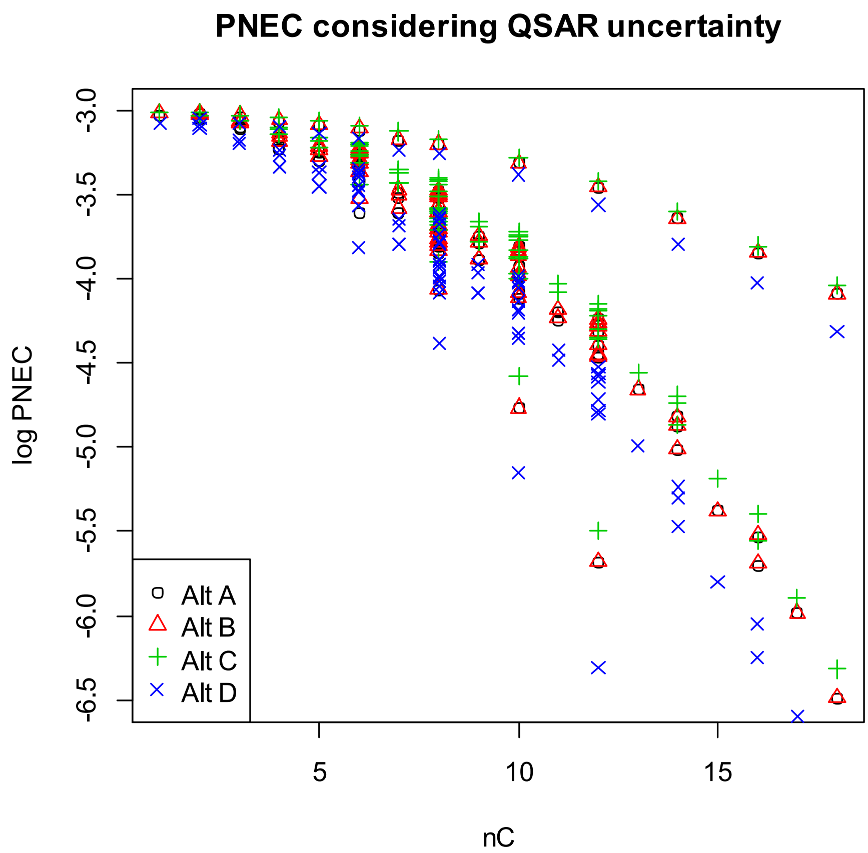


Figure 3. Hazardous concentration for four different treatments of uncertainty: Alt A. Conservative QSAR predictions, Alt B. Full uncertainty in QSAR predictions and 5th percentile of the output, Alt C. 5th percentile in fitted SSD based on conservative QSAR predictions, or Alt D. 5th percentile in fitted SSD based on double loop sampling from underlying predictive distributions of the QSAR predictions.

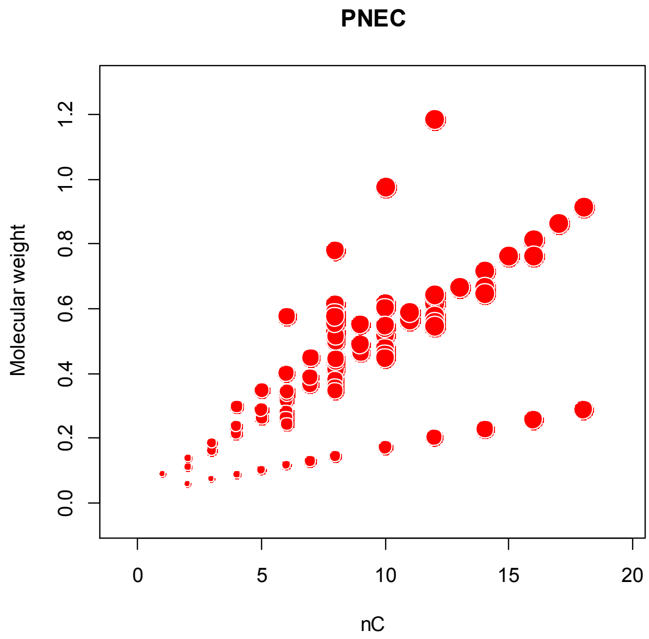


Figure 4. Relative hazards seen over length of carbon chain and molecular weight.

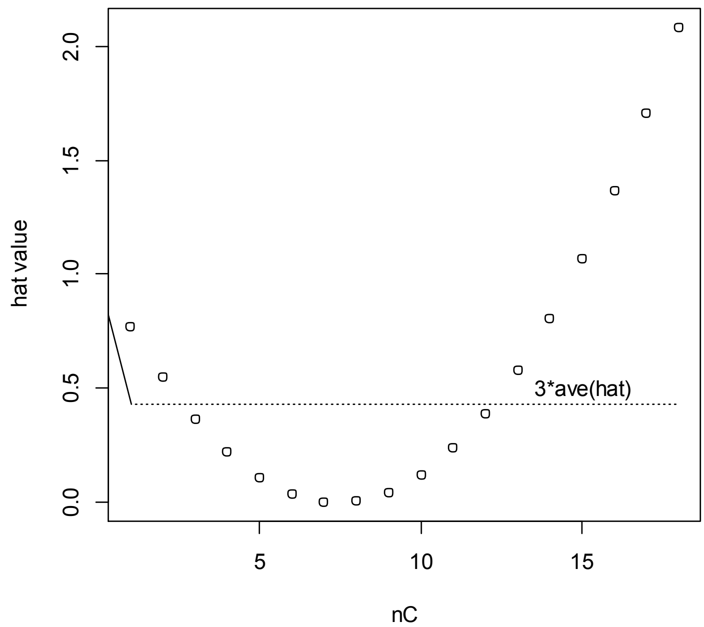


Figure 5. Predictive reliability evaluated by the distance to the model seen over different values on the single descriptor (nC) in the underlying QSARs for this hazard assessment.

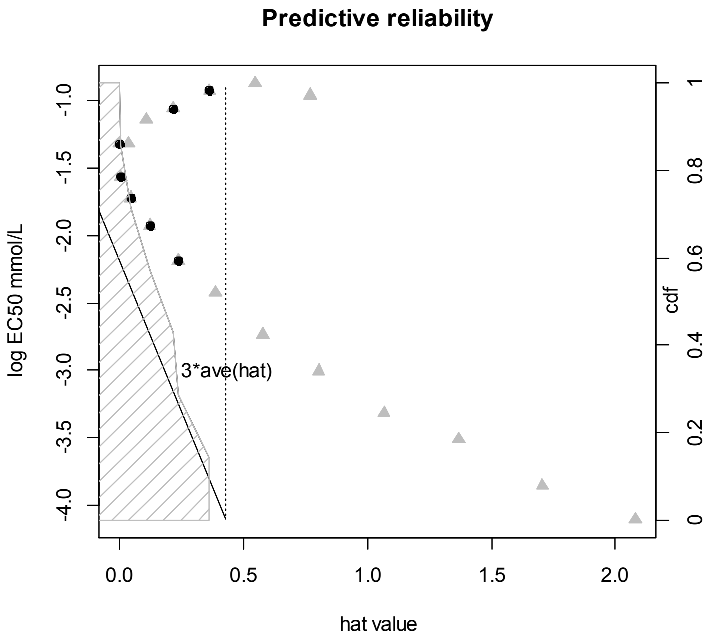


Figure 6. A graph suggested facilitating the communication of predictive reliability showing a metric of predictive reliability (distance to model evaluated by hat value) against QSAR prediction, and the positions of the training data set and predictions. The grey area is the descending cumulative distribution for the hat values of the training data set.

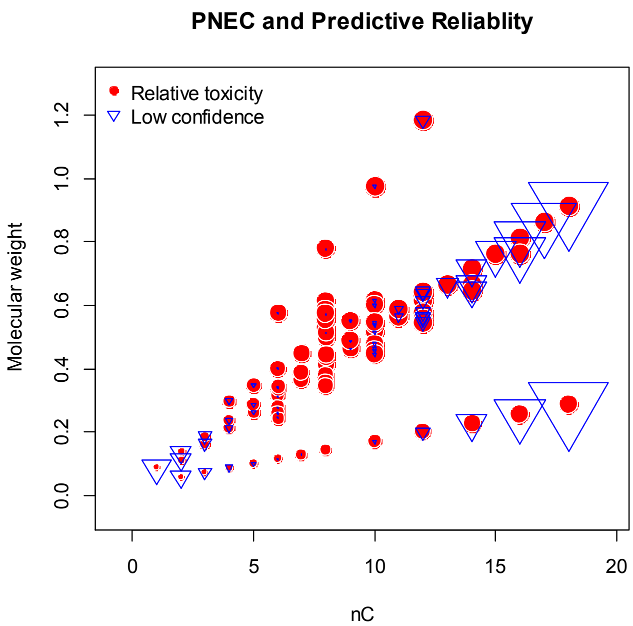


Figure 7. Relative hazards and relative confidence in predictions showed over two molecular characteristics.

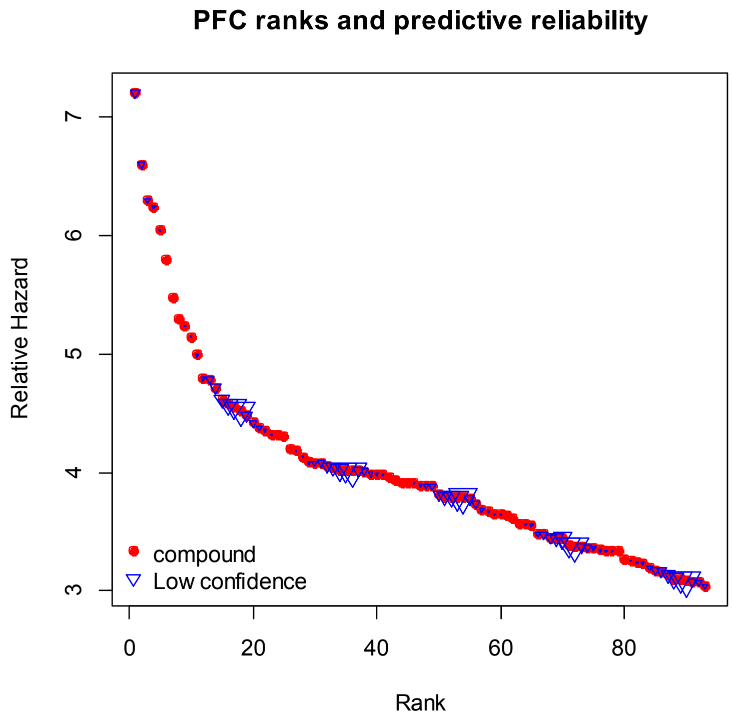


Figure 8. Ranking showing the relative confidence in predictions over the order of ranking based on relative hazards.

Table 1. Hazard assessment based on the AF method

Parameter	**Description**
Input:	
Min{ECwater}	The lowest valid effect concentration for organisms from the water compartment, i.e. EC50 or LC50 for short-term toxicity or EC10/NOEC for long term toxicity, typically given in mg/L or mg/kg
AF	Assessment factor, the size of which depends on the type and amount of toxicity information available
Output:	
PNECwater	Predicted No-Effect-Concentration for the compartment in question, typically given in mg/L or mg/kg

Table 2. Prioritization of PFCs based on relative hazards.

Compound Name	Relative Hazard (-log PNEC)	Rank
PFOcDA 18 (Perfluorooctadecanoic acid)	7.144	1
PFHpDA 17 (Perfluoroheptadecanoic acid)	6.678	2
PFHxDA 16 (perfluoro-n-hexadecanoic acid , 16)	6.236	3
10:2 diPAP	6.139	4
14:2 FTOH 1-Hexadecanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10, 10,11,11,12,12,13,13,14,14,15,15,16,16,16-nonacosafluoro-	6.037	5
PFPA, 15 (perfluoropentadecanoic acid)	5.771	6
PFTeA, 14 Perfluorotetradecanoic acid	5.416	7
12:2 FTOH 1-Tetradecanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10, 10,11,11,12,12,13,13,14,14,14-pentacosafluoro-	5.247	8
8:2 diPAP	5.212	9
12:2 FTolefin	5.186	10
PFTriA, 13 (perfluorotridecanoic acid)	4.991	11
10:2 PAP	4.709	12
n-C12F26	4.693	13
PFDoA, 12 Perfluorododecanoic acid	4.629	14
10:2 FTCA	4.534	15
10:2 FTOH 1-Dodecanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11,11,12,12,12-heneicosafluoro-	4.497	16
n-C11F24	4.496	17
10:2 FTUCA	4.481	18
10:2 FTolefin (1H,1H,2H Perfluoro-1-dodecene)	4.449	19
PFUnA, 11 Perfluoroundecanoic acid	4.435	20
Perfluorodecanesulfonic acid ammonium salt	4.396	21
6:2 diPAP	4.321	24
Stearic Acid, 18	4.292	25
8:2 PAP	4.233	26
n-C10F22	4.219	27

PFDcA, 10 Perfluorodecanoic acid	4.165	28
8:2 FTCA	4.083	31
8:2 FTOH 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-	4.052	32
MeFOSEA (N-methyl perfluotoocatane sulfonamido ethyl acrylat 25268-77-3)	4.04	33
8:2 FTUCA	4.038	34
hexadecanoic acid, 16	4.018	35
8:2 FTolefin (1H,1H,2H Perfluoro-1-decene)	4.011	36
n-C9F20	4.004	37
N-EtFOSAA (C8F17SO2N(CH2CH3)CH2COOH, 2-(N-ethyl perfluoro-1-octanesulfonamido)aceticacid)	3.996	38
PFNA, 9 Perfluorononanoic acid	3.954	41
N-MeFOSE (C8F17SO2N (C2H5O) C2H5, N-ethyl perfluorooctanesulfonamidoethanol, 24448-09-7)	3.948	42
t-Bu-PFOS (C8F17SO2NHC4H9, N-tert-butyl perfluorooctanesulfonamide)	3.945	43
N-EtFOSA (C8F17SO2NHC2H5, N-ethyl perfluorooctanesulfonamide, 4151-50-2)	3.897	44
3,7m2-PFOA (Perfluoro-3,7-dimethyl-octanoic acid)	3.875	45
N-MeFOSA (C8F17SO2NHCH3, N-methyl perfluoro-octanesulfonamide)	3.874	46
4:2 diPAP	3.858	47
PFSA Perfluorooctanesulfonamide	3.85	50
Myristic Acid, 14	3.771	51
6:2 PAP	3.756	52
n-C8F18	3.746	53
PFOA, 8 Perfluorooctanoic acid	3.705	56
6:2 FTCA	3.644	57
n-C7F16, Perfluoroheptane	3.628	58
6:2 FTOH 1-Octanol, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-	3.62	59
6:2 FTUCA	3.61	60
PFHpA, 7 Perfluoroheptanoic acid	3.589	63

6:2 FTolefin	3.589	64
Lauric Acid, 12	3.531	65
4:2 PAP	3.512	66
n-C6F14	3.503	67
PFHxA, 6 Perfluorohexanoic acid	3.468	68
4:2 FTCA	3.414	69
4:2 FTOH 1-Hexanol, 3,3,4,4,5,5,6,6,6-nonafluoro-	3.393	72
Decanoic Acid (Capric Acid), 10	3.39	73
4:2 FTUCA	3.384	74
4:2 FTolefin	3.366	75
n-C5F14	3.329	78
PFPeA, 5 (Perfluoropentanoic acid)	3.302	79
n-C4H10 DECAFLUOROBUTANE	3.263	80
Octanoic Acid (Caprylic Acid), 8	3.245	81
PFBA, 4 Perfluorobutanoic acid	3.236	82
Heptanoic acid	3.211	83
n-C3F8, Octafluoropropane (PFC-218)	3.203	84
PFPrA, Pentafluoropropionic acid, CF3CF2COOH	3.177	85
Hexanoic Acid, 6	3.173	86
n-C2F6, Hexafluoroethene	3.117	87
Pentanoic acid	3.117	88
Butanoic acid	3.097	89
TFA, trifluoroacetic acid, CF3COOH	3.097	90
Propionic acid	3.08	91
n-C1F4	3.072	92
Acetic Acid	3.051	93
PFDS, 10 (Perfluorodecane sulfonate)	4.36	22.5
PFDP, 10	4.36	22.5
PFNS, 9, Perfluorononane sulfonate	4.131	29.5
PFNP, 9	4.131	29.5
N-EtFOSE (C8F17SO2N (C2H5O) C2H5, N-ethyl perfluorooctanesulfonamidoethanol, 1691-99-2)	3.972	39.5

N-MeFOSAA (C8F17SO2N(CH3)CH2COOH, 2-(N-methylperfluoro-1-octanesulfonamido)aceticacid)	3.972	39.5
PFOS, 8	3.851	48.5
PFOP, 8	3.851	48.5
PFHpS, 7, Perfluoroheptane sulfonate	3.729	54.5
PFHpP, 7	3.729	54.5
PFHxS, 6, Perfluorohexane sulfonate	3.596	61.5
PFHxP, 6	3.596	61.5
PFPeS, 5, Perfluoropentane sulfonate	3.4	70.5
PFPeP, 5	3.4	70.5
PFBS, 4, Perfluorobutane sulfonate	3.331	76.5
PFBP, 4	3.331	76.5

Appendix 9

Case study 9: QSAR integrated fate assessment of PFCs

The Role of Uncertain K_{oc} Predictions in the Overall Persistency and Long Range Transport Potential of Perfluorinated Chemicals

INTRODUCTION

Perfluorinated chemicals (PFCs) are a group of chemicals of emerging concern, because they have been detected in the environment, in wildlife species, and in humans (ATSDR 2009). The family of perfluorinated chemicals includes perfluoroalkylated chemicals (like perfluorocarbon, perfluorobutane, perfluoropropane, and perfluorohexan), and their transformation products (like perfluoroalkylated sulfonamides, alkanoic acids, and sulfonates). Their appearance in the environment, animal species and humans is a consequence of their numerous applications and widespread use, in combination with their environmental persistence. The highest production volume is that of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). In addition, perfluorohexane sulfonic acid (PFHxS, a member of the same chemical class as PFOS); and perfluorononanoic acid (PFNA, a member of the same chemical class as PFOA) are produced in high quantities (ATSDR 2009). Examples of their use include packaging materials, waterproof and/or windproof clothing, fire-fighting foam, and non-sticking cookware. The combination of hydrophilic and lipophilic properties (so-called amphiphilic properties) makes them wanted chemicals in products to resist grease, oil, stains, and water. Furthermore, PFCs are resistant against acids, bases, oxidants, and reductants. Besides being extremely stable in industrial applications, they do not undergo metabolic transformation or other environmental degradation, because of their aggregated carbon–fluorine bonds (Environmental Working Group, 2003). Due to their potential for bioaccumulation they form a risk for ecosystem and human health. Experimental studies have shown a relationship between PFC exposure and an increased risk for liver toxicity (Seacat et al., 2002), developmental toxicity (Lau et al., 2003; Luebker et al., 2005; White et al., 2007; Fei et al., 2008), thyroid hormone disruption (Olsen et al., 2003; Chang et al., 2007), estrogenic effects (Bookstaff et al., 1990; Biegel et al., 1995; Butenhoff et al., 2002; Holm et al., 2003), infertility (Bonde et al., 1998; Fei et al., 2009; Joensen et al., 2009), immune system effects (Yang et al., 2000; Yang et al., 2001; Fairley et al., 2007), and elevated cholesterol levels (Sakr et al., 2007; Steenland et al., 2009; Nelson et al., 2010). Quantification of the environmental persistence and toxicity is essential for an assessment of the potential risks.

Despite of their emerging concern, PFCs are a group of data-poor chemicals (Ding and Peijnenburg, 2012). In the case of missing empirical data on physicochemical or toxic properties, predictions can be made with use of quantitative structure-activity relationships (QSARs) (Bhhatarai and Gramatica, 2010, 2011a, b; Bhhatarai et al., 2011). This way, an assessment of the potential risks can be facilitated. However, the uncertainty inherent in a QSARs prediction can have an effect on the outcome of the assessment. In this study, we quantified the uncertainty in the environmental fate related to the use of a QSAR for the organic carbon-water partitioning coefficient (K_{oc}). The K_{oc} is an important parameter in the environmental fate assessment of organic chemicals. Together with the fraction of organic carbon in the soil, it is used to derive the soil-water partitioning coefficient. This way it provides information on the extent of partitioning between soil and pore water, or between water and solid particles or sediment. Therefore, K_{oc} is an essential parameter for the determination of the overall persistence in the environment.

The goal of this study was to assess the influence of uncertainty in the QSAR prediction for K_{oc} on the fate assessment of perfluorinated chemicals. Environmental fate was assessed in terms of persistency and long range transport potential. The multimedia fate model Simplebox was used to estimate environmental fate of the PFCs. Uncertainties were treated as probability distributions, and propagated by Monte Carlo simulations based on the developed platform for QSAR-integrated assessment developed within the CADASTER project (Iqbal et al., 2012).

MATERIALS AND METHODS

Quantification of Environmental Fate. With the present modeling framework we assessed the environmental fate in terms of persistency and long range transport potential. There are several ways to quantify persistency in the environment and the potential for long range transport. A commonly used metric described for the overall persistency (P_{ov}) is the overall residence time of a chemical in the environment (days), which can be calculated according to Klasmeier et al. (2006):

$$P_{ov} = M_{tot} / E \tag{1}$$

where M_{tot} refers to the total steady state mass of the chemical in the environment (kg) and E to the total emission rate (kg/day).

The potential for long range transport (LRTP) can be defined as the (dimensionless) fraction of a chemical exported across the boundaries of the emitting region to a larger geographical area, calculated by:

$$LRTP = 1 – (M_R/M_{tot}) \tag{2}$$

where M_R is the total steady state mass of the chemical in the emitting region (kg). For chemicals with high LRTP this fraction is close to one; for chemicals with low LRTP the fraction is close to zero.

Prediction of K_{oc}. An experimental data set of 12 $\log K_{oc}$ values was provided by RIVM (Guanghui Ding, Willie J.G.M. Peijnenburg, Physicochemical Properties and Aquatic Toxicity of Poly- and Perfluorinated Compounds, accepted for publication in Reviews Environmental Science and Technology, 2013) and used for the development of regression models. This data set included 12 PFCs with different carbon chain lengths, fluorination degree and functional groups, including perfluorinated alkyl acids (PFAA), sulfonates (PFAS), sulfonamides and telomer alcohols (FTOH). For modelling purposes, the perfluorinated alkyl sulfonates (PFAS), were converted into the respective sulfonic acids (PFAS(A)). Since former studies (Papa et al., 2012; Kovarich et al., 2012) demonstrated that the salt-to-acid conversion did not influence the modelling results for these compounds (correct predictions for salts were obtained from acidic forms) PFASAs were included in the modelling dataset. Additional 45 heterogeneous PFCs, with unknown experimental $\log K_{oc}$, were added to the dataset, in order to generate a larger amount of predictions to be used for fate calculations (**Appendix Table A1**).

Over 470 Molecular descriptors were generated by DRAGON 5.5 starting from 3D structures created and energetically optimized by the semi-empirical method AM1 available in Hyperchem 7.Multiple linear regression models were developed by the ordinary least square regression method by the QSAR-INS software. All the possible combinations of the 473 input variables, in a population of models based on up to 2 descriptors, were generated. The models were optimized according to the cross validated Q^2 leave-one-out (Q^2_{LOO}). The robustness of the models was then evaluated by applying the Q^2leave-many-out (Q^2_{LMO}) with 50% perturbation of the original data set. Y-scrambling was also applied to verify that the models were not based on a chance correlation of descriptors with the response (low R^2_{SY} values calculated for models developed on scrambled responses, confirm the absence of chance correlation). The external validation of the models was not possible due to the extremely limited number of experimental data available.

In order to verify the ability of the proposed models to generate reliable predictions for new chemicals, the applicability domain (AD) was assessed taking into account the theoretical space defined by the descriptors used in the models. The AD was quantitatively defined by the leverage approach (Gramatica, 2007). The limit of a model domain was quantitatively defined by the leverage cut-off value (h^*): leverage values greater than h^* mean that the query compound is outside of the model structural AD. The applicability domain of the models was also graphically visualized in the plot "standardized errors vs. hat values" (Williams plot, **Figure 1**) and "predicted values vs. hat values" (Insubria Graph, **Figure 2**).

Multimedia Modeling. The PFCs' steady state fate and exposure were modeled in with the SimpleBox model, for default landscape settings (Den Hollander et al., 2004) . This is a fugacity based multimedia model in which the environment is modeled as a set of homogenous compartments; one compartment for each environmental medium in which the chemical is assumed to be evenly distributed. Results from SimpleBox are commonly used in EU risk assessments for new and existing chemicals (European Commission, 2003). We focused on the fate and potential for long range transport of PFC emissions of 1 kg/day to air, freshwater, and non-agricultural, non-natural soil on the regional scale.

Statistical Uncertainty. The experimental data underlying the multiple linear regressions and the descriptor domain of the training set (i.e. the set of chemicals used to develop the QSAR) were used to quantify the statistical uncertainty in the QSAR predictions. The uncertainty in a prediction Y_p from a QSAR being a multiple linear regression model fitted by the ordinary least squares method was assigned a Student-t distribution (Montgomery et al., 2001). The predictive Student-t distribution was defined by the predictive mean ($\overline{Y_p}$), the predictive error $SE(Y_p - \overline{Y_p})$, the number of chemicals in the training set (n), and the number of descriptors in the linear regression model (k), written as:

$$Y_p \sim \overline{Y_p} + t_{n-k-1} \cdot SE(Y_p - \overline{Y_p}) \quad (4)$$

where t_{n-k-1} stands for the *t*-distribution with n–k–1 degree of freedom.

Given that a QSAR was based on design matrix X, and Y_p was to be evaluated for descriptor values X_p, the predictive error was estimated as:

$$\left[SE(Y_p - \overline{Y_p})\right]^2 = s_r^{\,2}(1 + X_p^T (X^T X)^{-1} X_p) \quad (5)$$

It depended on the QSAR´s residual error (s_r), and the chemical-specific leverage value ($X_p^T (X^T X)^{-1} X_p$)(Atkinson, 1985; Mendenhall and Beaver, 1994; Montgomery et al., 2001). For chemicals in a QSAR's training set, the leverage value can be used to measure the chemical's influence on the QSAR model. However, leverage values can also be calculated for new compounds to indicate how far within or outside the applicability domain of the QSAR model a substance falls.

Monte Carlo Simulation. The uncertainties in the persistency and LRTP were determined with Monte Carlo simulations using the spreadsheet-based application Crystal Ball (Oracle©, Release 11.1.2.0.00) in MS Excel with 10,000 iterations per run.

Substance Selection. We selected 4 perfluorinated chemicals with different affinity for organic carbon, whose predicted fell within the applicability domain of the QSAR: 2-Perfluorooctyl ethanol (FTOH (8:2)); Perfluorooctane sulfonamide (FOSA); Perfluorohexanesulphonyl fluoride; and N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide. For details, see **Table 1**. These 4 PFCs are used to demonstrate the proof of principle, but the current modeling framework can also be applied to other PFCs.

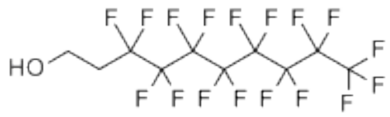
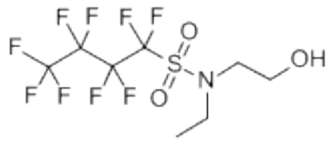
CAS	Name (abbreviation)	Chemical structure
000678-39-7	2-Perfluorooctyl ethanol (FTOH (8:2))	
000754-91-6	Perfluorooctane sulfonamide (FOSA)	
000423-50-7	Perfluorohexanesulphonyl fluoride	
034449-89-3	N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide	

Table 1. CAS-number, chemical name, abbreviation and chemical structure of the 4 substances modeled in this study

RESULTS AND DISCUSSION

Development of QSAR models for $LogK_{oc}$. A population of local QSAR models was generated from the 12 experimental $LogK_{oc}$ data. The equation and the statistical parameters of the best model are reported below:

$logK_{oc}$ =-3.1336 + 0.9927 X4 + 1.5295 nOHp
n tr: 12; Q^2_{LOO}: 0.8552; R^2: 0.9395; CCC tr: 0.9688;
Q^2_{LMO}: 0.7590 ; R^2_{SY}: 0.1749

This model has high fitting power and internal predictive ability, which was confirmed by Q^2_{LOO} value (>0.85) close to R^2 value, and $Q^2_{LMO50\%}$ about 0.8. The absence of chance correlation was checked with the Y-scrambling procedure and confirmed by small R^2_{YS} values.

The here proposed QSAR model was based on 2 molecular descriptors: the connectivity index (X4) and the number of primary alcohols (nOHp). Connectivity indices, which encode for molecular size and shape, have been already used as efficient descriptors to predict soil sorption coefficients (e.g. Meylan et al., 1992; Baker et al., 2001). The number of primary alcohols is relevant for the family of fluorotelomer alcohols (FTOHs), which are the volatile precursors of perfluorinated carboxylic acids.

The plot of experimental vs. predicted values, and the Williams plot for this model are reported in **Figure 1**. It is interesting to note that the variable selection procedure confirmed the importance of structural features which had been highlighted in literature (e.g. Meylan et al., 1992; Baker et al., 2001) as relevant for the modelling of $LogK_{oc}$.

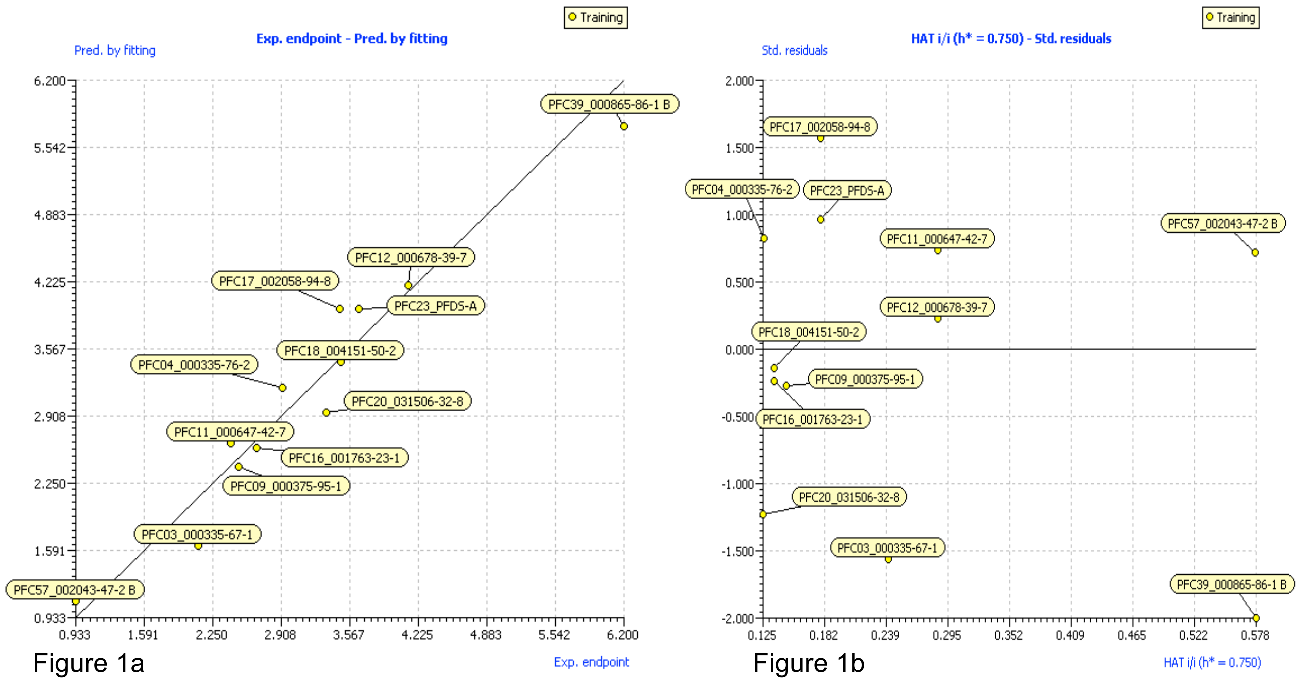


Figure 1. Plot of experimental vs. predicted values (Figure 1a), and Williams plot (Figure 1b), derived for the $LogK_{oc}$ model

None of the chemicals in the prediction set fell outside the structural applicability domain of the model; furthermore, the analysis of standardized residuals in prediction for all the chemicals in the training set did not highlight any response outlier (compound above or below the ±2.5 threshold values) (Figure 1b). The Insubria graph (Figure 2) shows that most of the 45 PFCs with unknown $LogK_{oc}$ (green points) are included in the applicability domain of the model which provides for these chemicals interpolated predictions. The 14 PFCs with predictions falling outside the applicability domain of the model are listed in the **Appendix Table A2**. These predictions should not be considered as reliable.

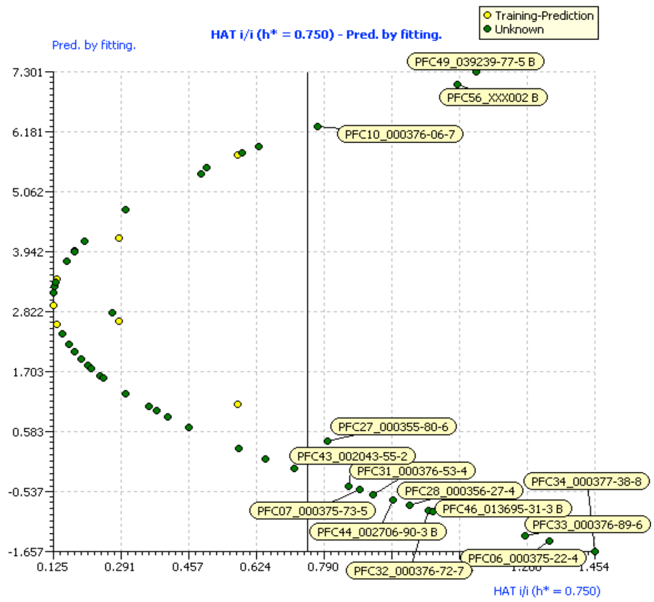


Figure 2. Plot of hat values vs. predicted values for 57 PFCs

However, even though the model proposed here is internally robust, and is based on relevant descriptors for the studied endpoint, its applicability is strictly dependent on the limited amount of data available to train the model. Additionally, since the external predictivity of the model could not be verified, predictions for any new chemical should be treated carefully and bearing in mind the "local" nature of this QSAR. Additional experimental data would be necessary to further verify the quality of the here proposed model.

Fate Predictions. We predicted organic carbon-water partitioning coefficients for four perfluorinated chemicals and assessed the influence of the QSAR uncertainty on the overall persistency and potential for long range transport. We assessed the influence of the QSAR uncertainty for an emission of 1 kg/day to air, freshwater and soil on a regional scale.

Typical values for overall persistency (i.e. median values) were found to range between 286 and 302 days for FTOH (8:2), depending on the emission compartment. The accompanying 95% confidence interval (95% CI) had a width of up to 97 days. For FOSA, typical P_{ov} values ranged between 549 and 608 days, with 95% CIs with a width of up to 318 days. For Perfluorohexane-sulphonyl fluoride, typical P_{ov} values ranged between 700 and 799 days, with 95% CIs of up to 62 days. For N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide, typical P_{ov} values ranged between 482 and 546 days, with 95% CIs of up to 343 days. The width of the confidence interval ranged from 8 percent of the typical value (Perfluorohexanesulphonyl fluoride), to 63 percent of the typical value (N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butane-sulfonamide).

When the PFC were emitted to air, typical values for the LRTP for the four PFCs ranged between 0.997 and 0.999 and uncertainty was negligible (≤ 0.1 percent of the typical value). For emissions to fresh water, typical values for the LRTP of the four PFCs ranged from 0.979 and 0.992, with small uncertainty (maximum 0.8 percent of the typical value). For emissions to soil, typical values for the LRTP of the four PFCs ranged from 0.954 and 0.983, with small uncertainty (maximum 1.7 percent of the typical value).

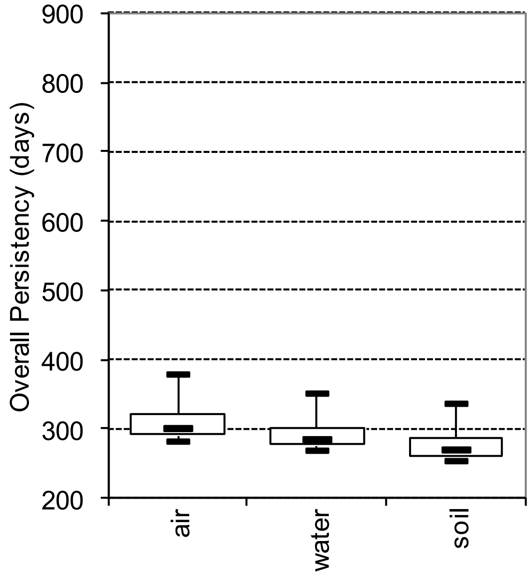
Table 2. Prediction of the log K_{oc} with accompanying predictive error, n is the number of chemicals in the QSAR's training set, s_r is the QSAR's residual error.

Chemical name (abbreviation)	**Pred. Log K_{oc}**	**$SE(Y_p - \overline{Y_p})$**	$n = 12$ $s_r = 0.121$
2-Perfluorooctyl ethanol (FTOH (8:2))	4.199	0.394	
Perfluorooctane sulfonamide (FOSA)	2.605	0.371	
Perfluorohexanesulphonyl fluoride	1.055	0.406	
N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide	2.821	0.392	

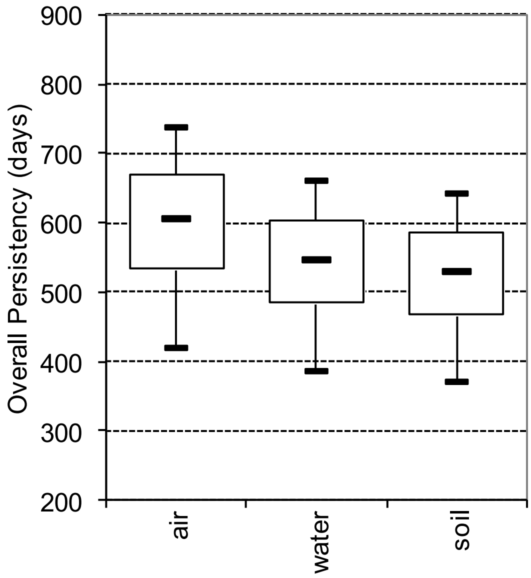
The predicted log K_{oc} values with their predictive errors are given in Table 2. The current results should be interpreted cautiously, since the QSAR was not validated due to data limitations. Another limitation of this study is the fact that we did not take into account dissociation. For neutral substances, the main determinants of the partitioning between water and organic carbon are the hydrophobic interactions between the chemical and the soil. For ionizing chemicals, however, electrostatic forces and the pH of the soil play a role in the sorption to particles. This is dependent on the acid dissociation constant of the substances (Franco and Trapp, 2008; Franco et al., 2009). Van Zelm et al. (2013) emphasized that for a proper assessment of the environmental fate of dissociating chemicals, the K_{oc} has to be determined for the mixture of neutral and ionic species.

We conclude that QSAR uncertainty in the organic carbon-water partitioning coefficient of PFCs has only small influence on the uncertainty in the potential for long range transport, but can have substantial influence on the uncertainty in the overall persistence. However, as dissociation was not taken into account, and data limitations prevented validation of the QSAR, the current results should be interpreted cautiously.

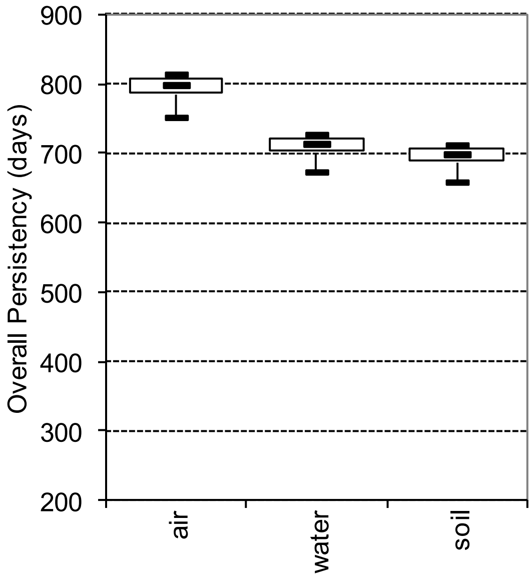
Figure 1. Box plots of the overall persistency (days) of FTOH (8:2), FOSA, Perfluorohexanesulphonyl fluoride, and N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide, for an emission of 1 kg/day to air, water and soil, respectively. The center of each box equals the median, the edges of each box represent the 25th and 75th percentile, and the whiskers the 5th and 95th percentiles.



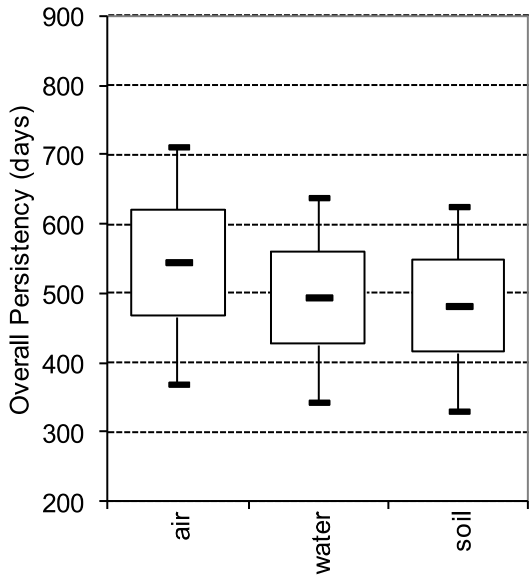
FTOH (8:2)



FOSA

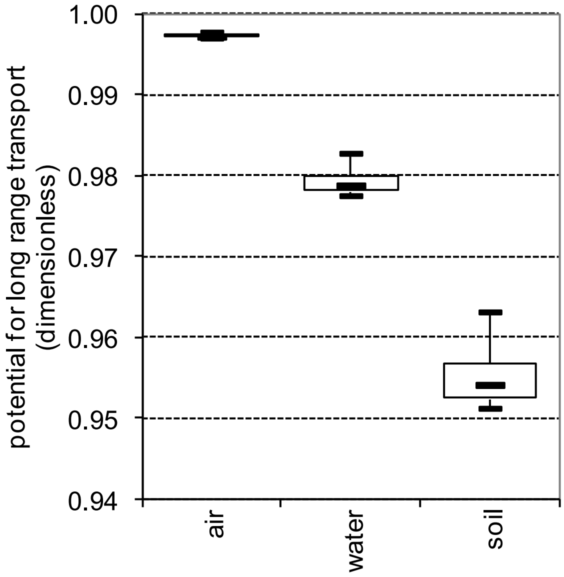


Perfluorohexanesulphonyl fluoride

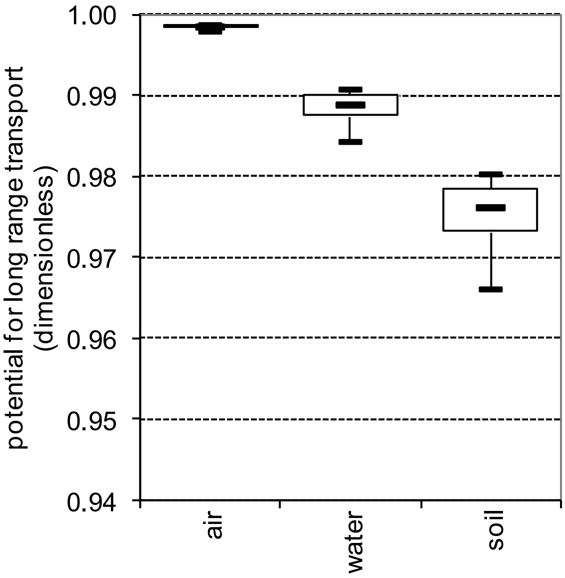


1-Butanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-

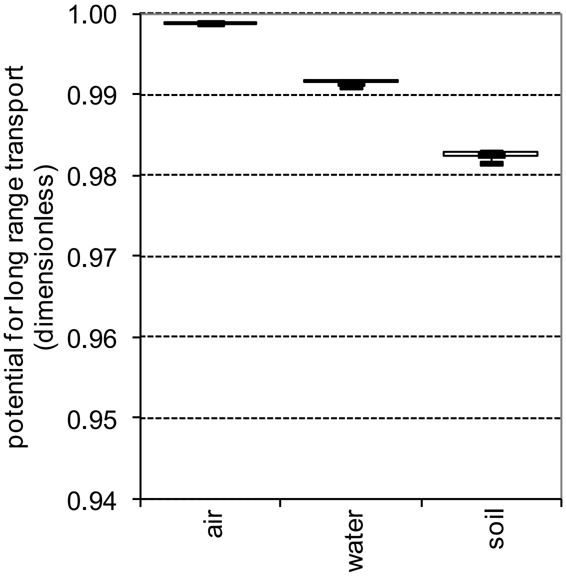
Figure 2. Box plots of the dimensionless potential for long range transport of FTOH (8:2), FOSA, Perfluorohexanesulphonyl fluoride, and N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-1-Butanesulfonamide, for an emission of 1 kg/day to air, water and soil, respectively. The center of each box equals the median, the edges of each box represent the 25th and 75th percentile, and the whiskers the 5th and 95th percentiles.



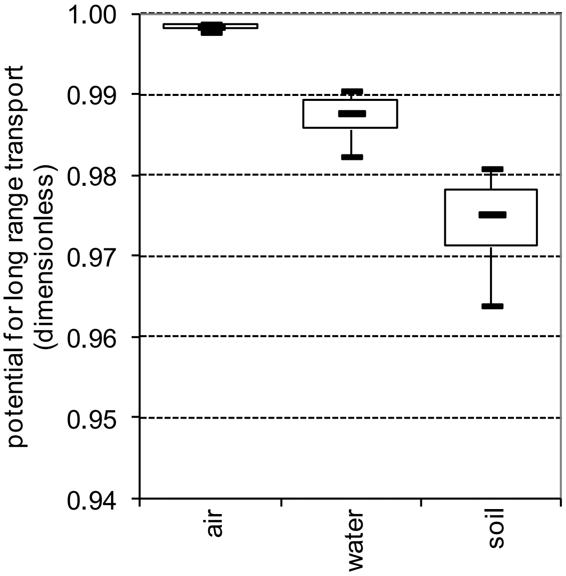
FTOH (8:2)



FOSA



Perfluorohexanesulphonyl fluoride



1-Butanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-

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APPENDIX to Appendix 9 – The Role of Uncertain K_{oc} Predictions in the Overall Persistency and Long Range Transport Potential of Perfluorinated Chemicals

Table A1. List of 57 PFCs included in the study. CAS (when available), names, experimental (when available) and predicted LogKoc values, hat values and values of the molecular descriptors included in the QSAR models (X4, and nOHp) are also reported.

ID	CAS, Abbreviation or Name	Name	Exp. LogKoc	Pred. LogKoc	HAT[a]	X4	nOHp
1	307-24-4	Perfluorohexanoic acid	-999	0.09	0.65	3.246	0
2	307-55-1	Perfluorododecanoic acid	-999	4.74	0.30	7.933	0
3	335-67-1	Perfluorooctanoic acid	2.11	1.64	0.24	4.808	0
4	335-76-2	Perfluorodecanoic acid	2.92	3.19	0.13	6.371	0
5	355-46-4	Perfluorohexane sulfonic acid	-999	1.05	0.36	4.219	0
6	375-22-4	Perfluorobutyric acid	-999	-1.46	1.3435 *	1.688	0
7	375-73-5	Nonafluorobutane sulfonic acid	-999	-0.50	0.88	2.656	0
8	375-85-9	Perfluoroheptanoic acid	-999	0.86	0.41	4.027	0
9	375-95-1	Perfluorononanoic acid	2.50	2.42	0.15	5.59	0
10	376-06-7	Perfluorotetradecanoic acid	-999	6.29	0.7741 *	9.496	0
11	647-42-7	2-Perfluorohexyl ethanol	2.43	2.65	0.29	4.283	1
12	678-39-7	2-Perfluorooctyl ethanol	4.13	4.20	0.29	5.846	1
13	754-91-6	Perfluorooctane sulfonamide	-999	2.61	0.14	5.781	0
14	1546-95-8	7H-Perfluoroheptanoic acid	-999	0.67	0.46	3.835	0
15	1691-99-2	2-(N-ethylperfluoro-1-octane sulfonamido) ethanol	-999	5.92	0.63	7.583	1
16	1763-23-1	Perfluorooctane sulfonic acid	2.68	2.61	0.14	5.781	0
17	2058-94-8	Perfluoroundecanoic acid	3.47	3.97	0.18	7.152	0
18	4151-50-2	N-ethyl perfluorooctane sulfonamide	3.49	3.44	0.14	6.627	0
19	24448-09-7	2-(N-methylperfluoro-1-octane sulfonamido) ethanol	-999	5.40	0.49	7.059	1
20	31506-32-8	N-methyl perfluorooctane sulfonamide	3.35	2.95	0.13	6.132	0
21	FTUA	2H-Perfluoro-2-octenoic acid (6:2)	-999	0.98	0.38	4.142	0
22	Me2FOSA	N,N-dimethyl perfluorooctane sulfonamide	-999	3.19	0.13	6.371	0
23	PFDS-A	Perfluorodecane sulfonic acid	3.66	3.97	0.18	7.152	0
24	PFOSi-A	Perfluorooctane sulfinic acid	-999	2.42	0.15	5.59	0
25	76-21-1	Hexadecafluoro-nonanoic acid	-999	2.22	0.16	5.398	0

26	336-08-3	Hexanedioic acid, 2,2,3,3,4,4,5,5-octafluoro-	-999	-0.10	0.72	3.054	0
27	355-80-6	1-Pentanol, 2,2,3,3,4,4,5,5-octafluoro-	-999	0.42	0.7986 *	2.036	1
28	356-27-4	Butanoic acid, heptafluoro-, ethyl ester	-999	-0.78	1.0003 *	2.376	0
29	375-81-5	Perfluoropentane-1-sulphonyl fluoride	-999	0.28	0.58	3.438	0
30	375-92-8	Pentadecafluoro-1-heptanesulfonic acid	-999	1.83	0.21	5	0
31	376-53-4	Adiponitrile, perfluoro	-999	-0.58	0.9112 *	2.576	0
32	376-72-7	Octafluoropentanoic acid	-999	-0.88	1.0476 *	2.274	0
33	376-89-6	Hexafluoroglutaronitrile	-999	-1.35	1.2838 *	1.8	0
34	377-38-8	Perfluorosuccinic acid	-999	-1.66	1.4539 *	1.488	0
35	423-50-7	Perfluorohexanesulphonyl fluoride	-999	1.05	0.36	4.219	0
36	423-54-1	Perfluorooctanamide	-999	1.64	0.24	4.808	0
37	559-11-5	1,1-Dihydroperfluoroheptyl acrylate	-999	1.30	0.30	4.47	0
38	756-91-2	3-Penten-1,5-diol, 3-methyl-1,1,5,5-tetrakis(trifluoromethyl)-	-999	1.77	0.22	4.941	0
39	865-86-1	1,1,2,2-Tetrahydroperfluoro dodecanol	6.20	5.75	0.58	7.408	1
40	918-21-8	Perfluoropinacol	-999	2.08	0.18	5.25	0
41	1478-61-1	Hexafluoroacetone bisphenol A	-999	4.15	0.20	7.339	0
42	1765-48-6	11-Eicosafluoroundecanoic acid	-999	3.78	0.16	6.96	0
43	2043-55-2	Hexane, 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodo-	-999	-0.43	0.8496 *	2.721	0
44	2706-90-3	Perfluoropentanoic acid	-999	-0.69	0.9601 *	2.465	0
45	2706-91-4	Perfluoropentanesulfonic acid	-999	0.28	0.58	3.438	0
46	13695-31-3	Heptafluorobutyl methacrylate	-999	-0.90	1.059 *	2.25	0
47	17527-29-6	1,1,2,2-Tetrahydroperfluorooctyl acrylate	-999	1.59	0.25	4.755	0
48	34449-89-3	1-Butanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,4-nonafluoro-N-(2-hydroxyethyl)-	-999	2.82	0.27	4.458	1
49	39239-77-5	1,1,2,2-Tetrahydroperfluoro-1-tetradecanol	-999	7.30	1.1627 *	8.971	1
50	57729-98-3	Benzenamine, N-(2,4-difluorophenyl)-2,4-dinitro-6-(trifluoromethyl)-	-999	3.31	0.13	6.494	0
51	65592-51-0	Benzenamine, N-methyl-2,4-dinitro-6-(trifluoromethyl)-N-(3-(trifluoromethyl)phenyl)-	-999	3.95	0.18	7.139	0
52	67939-33-7	2-Propenoic acid, 2-methyl-, 2- ethyl (nonafluorobutyl)sulfonyl amino ethyl ester)	-999	1.96	0.19	5.129	0
53	68259-12-1	Nonadecafluoro-1-nonanesulfonic acid	-999	3.38	0.13	6.563	0
54	107350-42-5	Cyclohexanecarboxamide, 1,2,2,3,3,4,4,5,5,6,6-	-999	5.79	0.59	8.989	0

		undecafluoro-N-(2,3,4,5-tetrachlorophenyl)-					
55	Perfluorotridecanoic acid	Perfluorotridecanoic acid	-999	5.52	0.50	8.715	0
56	Perfluoropentadecanoic acid	Perfluoropentadecanoic acid	-999	7.07	1.1184 *	10.277	0
57	002043-47-2	2-Perfluorobutylyl ethanol	0.93	1.10	0.58	2.721	1

Table A2. List of PFCs out of the applicability domain of the model

ID	CAS/Abbreviation	Name	LogKoc Pred.	HAT
6	375-22-4	Perfluorobutyric acid	-1.46	1.3435
7	375-73-5	Nonafluorobutane sulfonic acid	-0.50	0.8769
10	376-06-7	Perfluorotetradecanoic acid	6.29	0.7741
27	355-80-6	1-Pentanol, 2,2,3,3,4,4,5,5-octafluoro-	0.42	0.7986
28	356-27-4	Butanoic acid, heptafluoro-, ethyl ester	-0.78	1.0003
31	376-53-4	Adiponitrile, perfluoro	-0.58	0.9112
32	376-72-7	Octafluoropentanoic acid	-0.88	1.0476
33	376-89-6	Hexafluoroglutaronitrile	-1.35	1.2838
34	377-38-8	Perfluorosuccinic acid	-1.66	1.4539
43	2043-55-2	Hexane, 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodo-	-0.43	0.8496
44	2706-90-3	Perfluoropentanoic acid	-0.69	0.9601
46	13695-31-3	Heptafluorobutyl methacrylate	-0.90	1.059
49	39239-77-5	1,1,2,2-Tetrahydroperfluoro-1-tetradecanol	7.30	1.1627
56	PFPA	Perfluoropentadecanoic acid	7.07	1.1184

Appendix 10

Description of the Webtool for risk assessment of chemical compounds

Introduction and technical details

The developed web tool is an implementation of Environmental Probabilistic Risk Assessment specifically designed to integrate QSAR predictions. The work was initiated by collaboration between WP4 and WP5 in the CADASTER project. The initial development of the webtool was performed in the group of Dr. Tetko during the MC ITN fellowship of Mr. Sopasakis[2] and after that continued within the CADASTER project. The framework is based on a R-wrapper (initially provided by Dr. Ullrika Sahlin LnU) around SimpleBox[3] code developed by the RIVM scientists (processed for QSAR integrated assessment for BDEs, Triazoles and PFC as indicated in section 3). The initial results were presented during the Second CADASTER Workshop, which took place during October 7-9 in Neuherberg, Germany.

During the last quarter of 2012, a new implementation of the calculation servers and the graphical interface was developed by Dr. Tetko and Mr. Kunwar. Mr. Brandmaier, contributed effect assessment through the calculation of PNEC values based on the Species Sensitivity Distribution approach. The underlying R-code was provided by Dr. Ulrika Sahlin LnU. QSAR integrated fate and effect assessment were linked to developed models that predict physico-chemical properties and toxicity values of molecules. Another important input for risk assessment is the rate of emission into the selected environmental compartments and scale of the Simple box models (details found in associated articles). Since several models developed by CADASTER partners were based on SRC EPI suite[4], we implemented an interface to this package.

Physico-chemical parameters required by the webtool

All physico-chemical parameters have been separated in two blocks: essential and optional ones. The parameters, which are essential ones for the data analysis, as well as their units are provided in Table 1.

Table 1. List of essential parameters identified for the Fate Assessment.

Property Name	Variable Name	Unit
Water SOLUBILITY at 25 °C	Sol25	log(mg/L)
Melting point	Tm	[oC]C
VAPOR PRESSURE at 25 °C	Pvap25	[Pa]
Partition coeff., organic carbon/water at 25°C.	KOC	[-]
Dissolved phase degradation RATE CONSTANT at 25 °C	kdeg.water	[s-1]
Bulk degradation RATE CONSTANT standard sediment at 25 °C	kdeg.sed	[s-1]
Bulk degradation RATE CONSTANT standard soil at 25 °C	kdeg.soil	[s-1]
Gas phase degradation RATE CONSTANT at 25 °C	kdeg.air	[s-1]
Octanol/water PARTITION COEFFICIENT	Kow	[-]

These parameters are essential to be specified by the user for the Fate Assessment. In addition, there is also a list of optional parameters, which could be used as predefined values or/and calculated using the other parameters.

Table 2. List of optional parameters for the Fate Assessment.

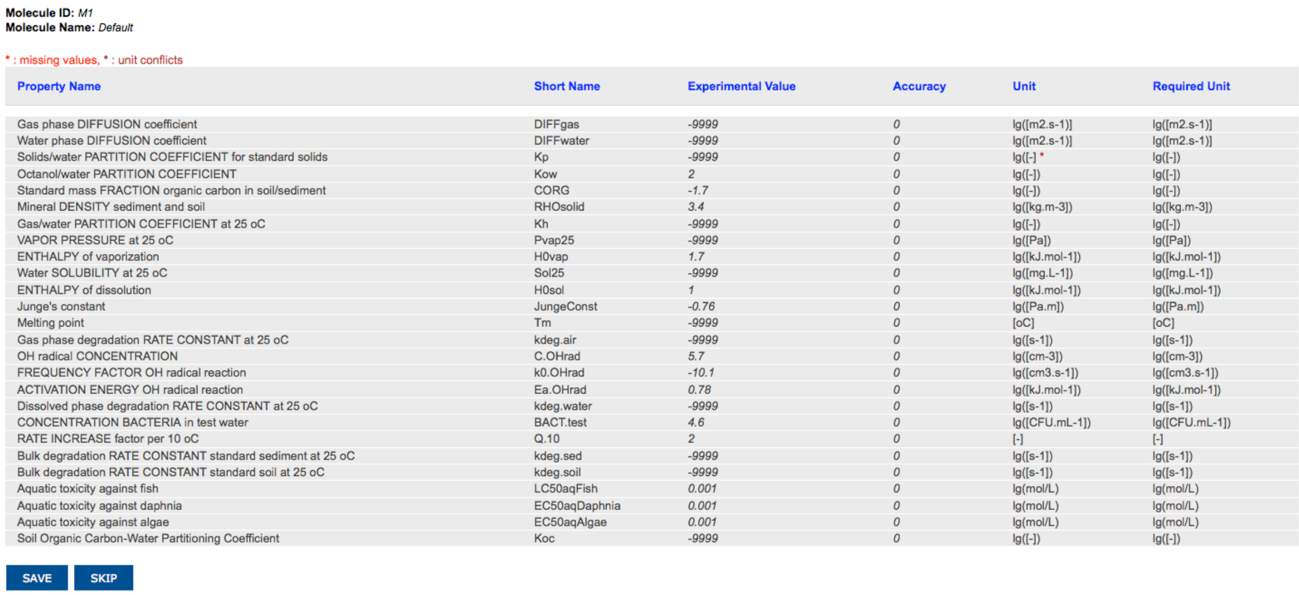
Standard mass FRACTION organic carbon in soil/sediment	CORG	Units	0.02
Mineral DENSITY sediment and soil	RHOsolid	[kg.m-3]	2500
ENTHALPY of vaporization	H0vap	[kJ.mol-1]	50
ENTHALPY of dissolution	H0sol	[kJ.mol-1]	10
Junge's constant	JungeConst	[Pa.m]	0.172
OH radical CONCENTRATION	C.OHrad	[cm-3]	500000
FREQUENCY FACTOR OH radical reaction	k0.OHrad	[cm3.s-1]	7.9E-11
ACTIVATION ENERGY OH radical reaction	Ea.OHrad	[kJ.mol-1]	6
CONCENTRATION BACTERIA in test water	BACT.test	[CFU.mL-1]	40000
RATE INCREASE factor per 10 ^{o}C	Q.10	[-]	2
Photocatalytic degradation rate constant at 25°C	Kphoto	[s-1]	0.001

The default values for these parameters were calculated for the class of PBDE, using Dibutilamina 2,2',3,4,4',5',6-heptabromodiphenyl ether as the target. It is obvious that the extrapolation and use of these default values for other classes of compounds could result in an improper risk assessment.

However, the users can upload his/her own experimental or calculated values for the compound, which will be used in the Fate assessment. This can be done in several ways:

Firstly (and preferred) the user can upload the values to the QSP-THESAURUS database. In this case the values can be stored in the database together with the reference and proper acknowledgement of the data source of the publication.

Secondly, the user can prepare all parameters for the fate assessment in one file and upload them to the database. An example of the upload page is shown on Figure 1.



Molecule ID: *M1*
Molecule Name: *Default*

* : missing values, * : unit conflicts

Property Name	Short Name	Experimental Value	Accuracy	Unit	Required Unit
Gas phase DIFFUSION coefficient	DIFFgas	-9999	0	lg([m2.s-1)]	lg([m2.s-1)]
Water phase DIFFUSION coefficient	DIFFwater	-9999	0	lg([m2.s-1)]	lg([m2.s-1)]
Solids/water PARTITION COEFFICIENT for standard solids	Kp	-9999	0	lg([-] *	lg([-])
Octanol/water PARTITION COEFFICIENT	Kow	2	0	lg([-])	lg([-])
Standard mass FRACTION organic carbon in soil/sediment	CORG	-1.7	0	lg([-])	lg([-])
Mineral DENSITY sediment and soil	RHOsolid	3.4	0	lg([kg.m-3])	lg([kg.m-3])
Gas/water PARTITION COEFFICIENT at 25 oC	Kh	-9999	0	lg([-])	lg([-])
VAPOR PRESSURE at 25 oC	Pvap25	-9999	0	lg([Pa])	lg([Pa])
ENTHALPY of vaporization	H0vap	1.7	0	lg([kJ.mol-1])	lg([kJ.mol-1])
Water SOLUBILITY at 25 oC	Sol25	-9999	0	lg([mg.L-1])	lg([mg.L-1])
ENTHALPY of dissolution	H0sol	1	0	lg([kJ.mol-1])	lg([kJ.mol-1])
Junge's constant	JungeConst	-0.76	0	lg([Pa.m])	lg([Pa.m])
Melting point	Tm	-9999	0	[oC]	[oC]
Gas phase degradation RATE CONSTANT at 25 oC	kdeg.air	-9999	0	lg([s-1])	lg([s-1])
OH radical CONCENTRATION	C.OHrad	5.7	0	lg([cm-3])	lg([cm-3])
FREQUENCY FACTOR OH radical reaction	k0.OHrad	-10.1	0	lg([cm3.s-1])	lg([cm3.s-1])
ACTIVATION ENERGY OH radical reaction	Ea.OHrad	0.78	0	lg([kJ.mol-1])	lg([kJ.mol-1])
Dissolved phase degradation RATE CONSTANT at 25 oC	kdeg.water	-9999	0	lg([s-1])	lg([s-1])
CONCENTRATION BACTERIA in test water	BACT.test	4.6	0	lg([CFU.mL-1])	lg([CFU.mL-1])
RATE INCREASE factor per 10 oC	Q.10	2	0	[-]	[-]
Bulk degradation RATE CONSTANT standard sediment at 25 oC	kdeg.sed	-9999	0	lg([s-1])	lg([s-1])
Bulk degradation RATE CONSTANT standard soil at 25 oC	kdeg.soil	-9999	0	lg([s-1])	lg([s-1])
Aquatic toxicity against fish	LC50aqFish	0.001	0	lg(mol/L)	lg(mol/L)
Aquatic toxicity against daphnia	EC50aqDaphnia	0.001	0	lg(mol/L)	lg(mol/L)
Aquatic toxicity against algae	EC50aqAlgae	0.001	0	lg(mol/L)	lg(mol/L)
Soil Organic Carbon-Water Partitioning Coefficient	Koc	-9999	0	lg([-])	lg([-])

SAVE SKIP

Figure 1. The interface to upload physico-chemical and toxicity properties of molecules to the QSPR-Thesaurus database.

The molecule uploaded this way and its property values will be accessible in the section "Provided values" of the Risk Assessment interface. The user can update information for the same molecule by uploading the data for the same molecule again.

Workflow for calculation of fate assessment

The available workflow was developed for the emission scenario of molecules in the atmosphere and was developed for PBDE. The emission scenario could be different for other classes of molecules, e.g. triazoles and benzo-triazoles, which are actively used as pesticides and enter the environment via various pathways.

The first step of the analysis starts with providing the target molecule. The user can either draw or select it from the list of previously uploaded molecules, for which the user provided physico-chemical and toxicity values.

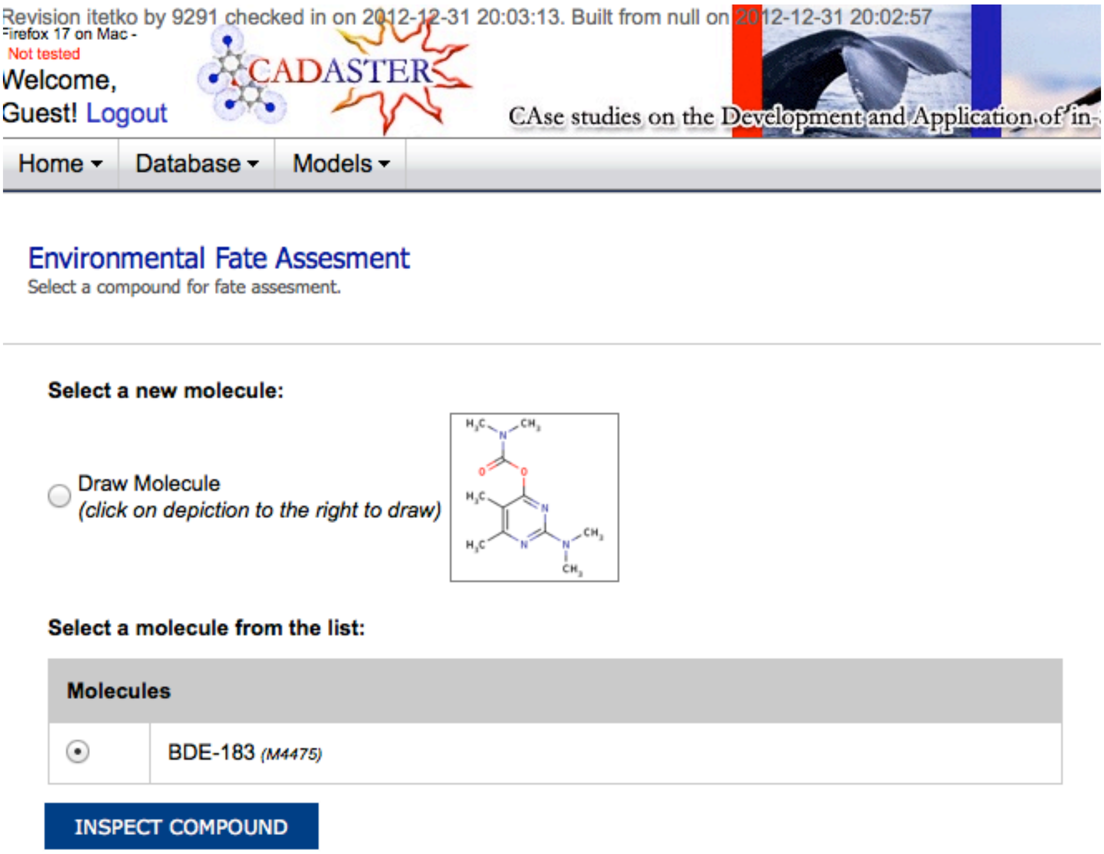


Figure 2. The interface to select the molecules for the risk assessment. In this example the user selected BDE-183 (Dibutilamina ; CCRIS 3645 ; 2,2',3,4,4',5',6-heptabromodiphenyl ether) for further analysis. The user can also draw molecule using available Java editor.

Following this, the user proceeds to the next page, which contains information about the values of the molecules. The web tool examines whether the QSPR THESAURUS database contains experimental values for the analyzed molecules. In case it does, it retrieves all such values, converts them to default values and displays all these values as shown in Figure 3.

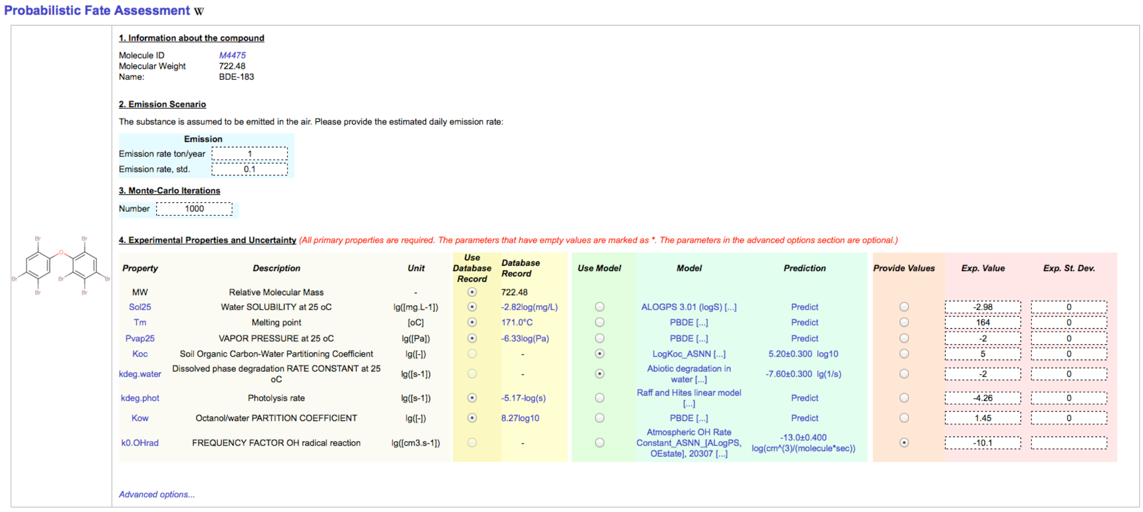


Figure 3. The web-page with experimental, predicted and user-specified physico-chemical parameters of the molecules.

In some cases the database may contain several experimental values for the same property and molecule. Given this situation, the user can examine all available values.

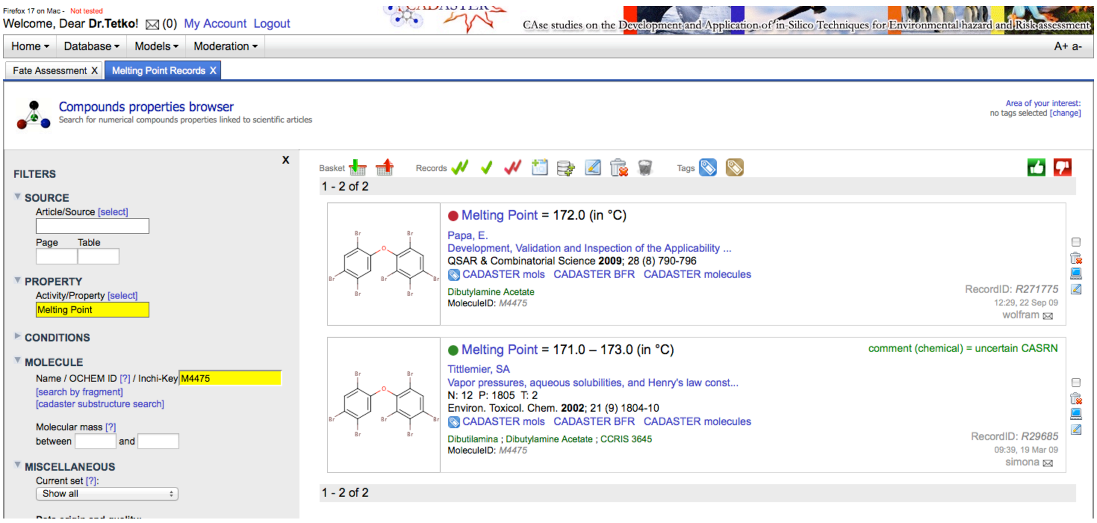
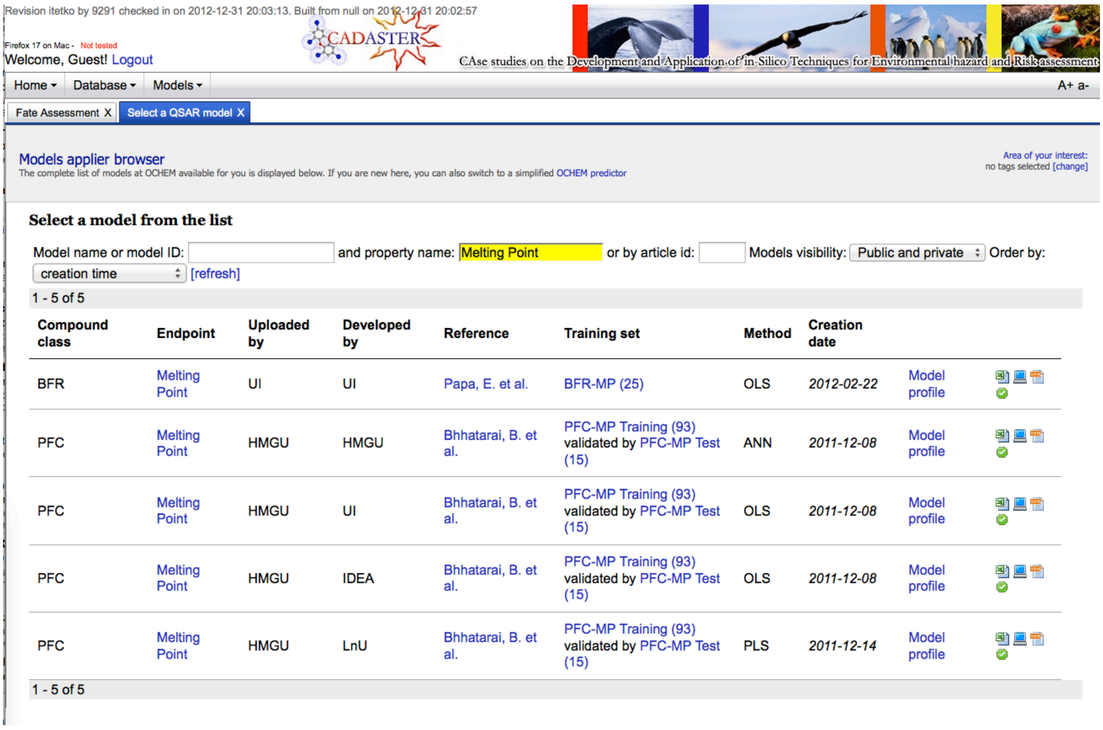


Figure 4. The web interface to the experimental properties of molecules. The user can inspect which experimental values are available in the database and their source.

The QSPR-THESAURUS database contains a number of QSAR and QSPR models for the analyzed physico-chemical properties. These models were uploaded and published on the web site by the CADASTER project team. For such properties, the use of those models is proposed to the user.



Compound class	Endpoint	Uploaded by	Developed by	Reference	Training set	Method	Creation date		
BFR	Melting Point	UI	UI	Papa, E. et al.	BFR-MP (25)	OLS	2012-02-22	Model profile	
PFC	Melting Point	HMGU	HMGU	Bhhatarai, B. et al.	PFC-MP Training (93) validated by PFC-MP Test (15)	ANN	2011-12-08	Model profile	
PFC	Melting Point	HMGU	UI	Bhhatarai, B. et al.	PFC-MP Training (93) validated by PFC-MP Test (15)	OLS	2011-12-08	Model profile	
PFC	Melting Point	HMGU	IDEA	Bhhatarai, B. et al.	PFC-MP Training (93) validated by PFC-MP Test (15)	OLS	2011-12-08	Model profile	
PFC	Melting Point	HMGU	LnU	Bhhatarai, B. et al.	PFC-MP Training (93) validated by PFC-MP Test (15)	PLS	2011-12-14	Model profile	

Figure 5. A list of models available for the Melting Point. "Compound class" indicates CADASTER class of molecules (PFC, BFR, TAZ& BTAZ and fragrances) for which the model was developed. The user is asked to select those models that were developed for the molecule. In case there are no models developed for the particular class of molecules, the user may use a model, e.g. KOWWIN or ALOGPS for logP, which was developed for a wide range of chemical compounds.

Once the appropriate models have been chosen, the QSAR models are applied to the specific compounds of interest. At the end of the calculations the predicted values and their predicted errors are provided. These values can be used for the Fate Assessment of the analyzed molecule.

In case no models are available or if they are not applicable, the user can introduce data and the measurement errors of data (if available) in the "User provided values" section of the interface. The data should be introduced in the pre-defined units.

Finally, the Emission rate (tons/year), the according standard deviation, as well as the number of required number of iterations for the Monte Carlo simulations have to be specified.

Once all information is provided, the user can start calculations by clicking the "Calculate" button. The Webtool collects all the data and proceeds to the next page.

Environmental Fate Assessment W

Inspect the parameters

Parameter	Source	Value	Uncertainty
Kow	DatabaseRecord	8.27	0.0
CORG	ExperimentalProperty	0.02	-
RHOsolid	ExperimentalProperty	3.4	-
Pvap25	DatabaseRecord	-6.33	0.0
H0vap	ExperimentalProperty	1.7	-
Sol25	DatabaseRecord	-2.82	0.0
H0sol	ExperimentalProperty	1.0	-
JungeConst	ExperimentalProperty	-0.76	-
Tm	DatabaseRecord	171.0	0.0
kdeg.phot	DatabaseRecord	-5.17	0.0
C.OHrad	ExperimentalProperty	5.7	-
k0.OHrad	ExperimentalProperty	-10.1	-
Ea.OHrad	ExperimentalProperty	0.78	-
kdeg.water	QSARModel	-7.6	0.3
BACT.test	ExperimentalProperty	4.6	-
Q.10	ExperimentalProperty	2.0	-
Koc	QSARModel	5.2	0.3

Download results Continue with hazard estimation

Figure 6. Preview of physico-parameters submitted for the calculations to SimpleBox.

The provided parameters are submitted for calculation to the SimpleBox. Monte-Carlo calculations are provided to simulate the uncertainty within the provided parameters.

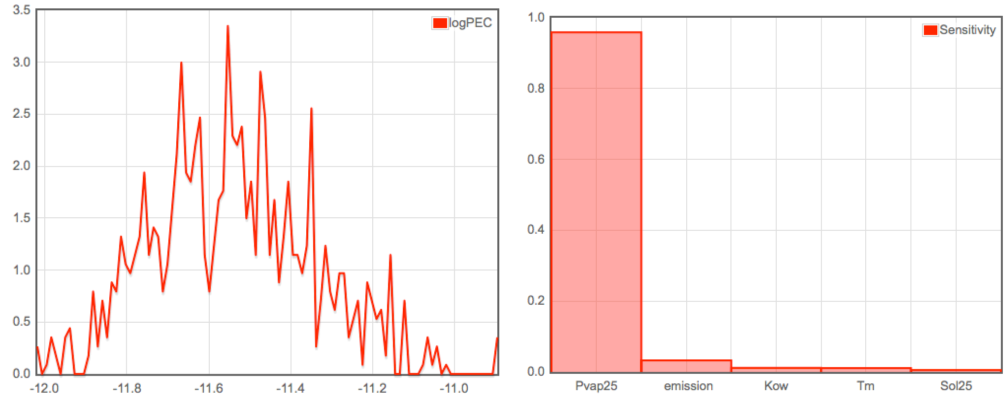


Figure 7. The left panel shows the distribution of PEC values calculated using SimpleBox. The right panel indicates the contribution of different physico-chemical parameters on the resulting PEC values. The calculations were performed using N=1000 iterations.

An optimization to speed up Monte Carlo simulations was done. Usually, in Monte Carlo simulations the input values are simultaneously drawn from respective distributions. Thus all values of the input parameters, which are used in the calculations, could be changed on the same iteration. The current implementation of calculations inside the Excel sheet is based on the RDCOMClient library provided by the Omega project for scientific computing (http://www.omegahat.org/RDCOMClient). This implementation is using asynchronous updates of the values in the Excel sheet. After updating of each value, Excel starts to recalculate all values in the sheet. Thus the speed of calculations linearly decreases with the number of input values to be used in Monte Carlo simulations. In order to overcome this limitation, only one value for each input variable was changed within each Monte Carlo simulation. This implementation dramatically increased the speed of calculations.

Figure 7 shows that for the emission to the air scenario, the highest influence on the distribution of PEC values is provided by the model for the vapor pressure. This result is very logical, since this physico-chemical parameter is decisive for the fate of chemicals in the air.

The use of this Webtool therefore supports the discrimination into highly relevant parameters and insignificant ones for the estimation of PEC values. The shape of the distribution on Figure 7 (left panel) is noisy. More smooth estimations can be provided using an increased number of iterations (e.g. 10000).

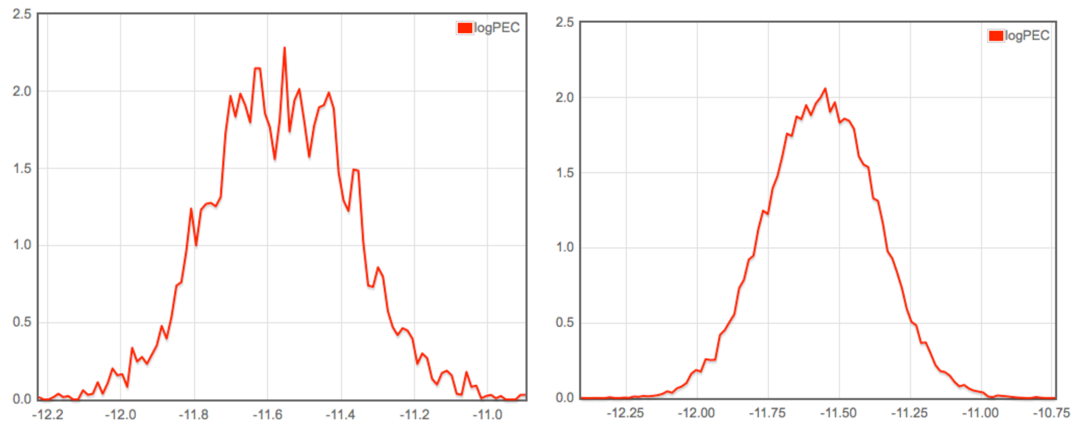


Figure 8. The PEC distribution calculated using N=10,000 and N=100,000 iterations. The shape of the curve becomes smoother with increasing N. However, using a high number of iterations requires proportionally a longer time (linearly dependency regarding N). In case of the given example and 100k iterations the calculation required about 2 hours to complete. This problem can be addressed by parallelizing calculations on several computers.

All values used as input for SimpleBox Monte Carlo calculations and distributions of PEC, Persistency and LRTP values are available for download.

Workflow of calculation of Effect assessment

Effects were assessed as Predicted No Effect Concentration (PNEC) values into the same environment as the output variables of the Fate assessment were taken from, in this case the aquatic environment. Once PEC calculations are running, the user can immediately start with the Species Sensitivity Distribution analysis. The user can also perform the hazard assessment directly without a need to perform the fate assessment from the main menu of the QSPR Thesaurus database.

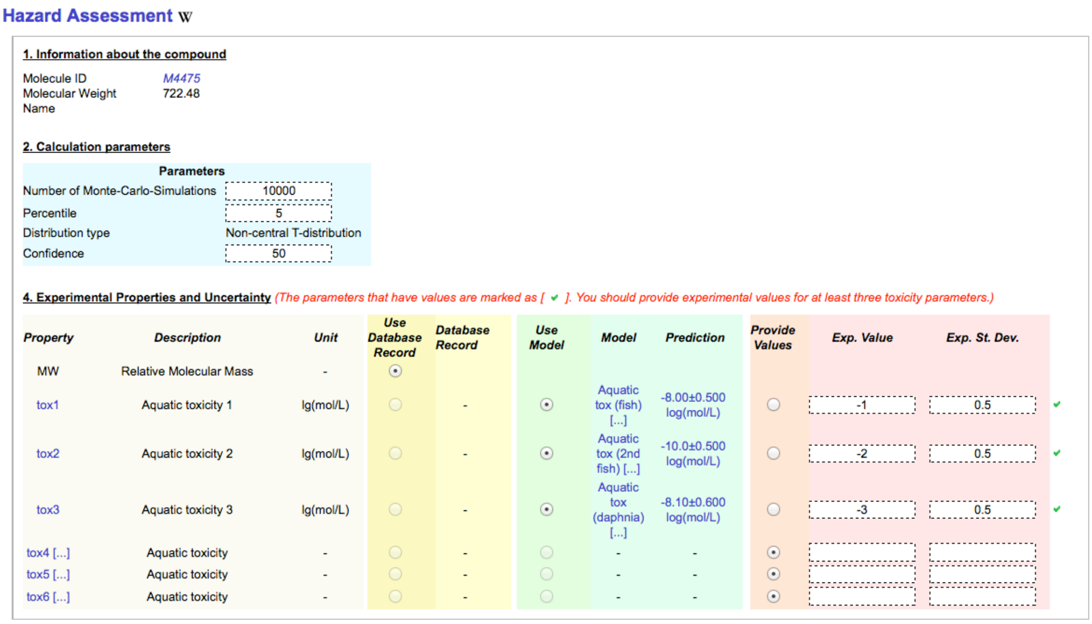


Figure 9. The interface to introduce toxicity data for the assessment of hazardous concentration using the Species Sensitivity Distribution approach. The design of interface is similar to that for the Fate Assessment. The user can use experimental toxicity values, calculate them with models or/and provide expert knowledge. Toxicity values are required to be provided at least for three species. The user may use pre-selected models or/and select new ones.

There are three predefined species, namely Fish, Daphnia and Algae, for which prediction models are provided. The models for these species were developed by the CADASTER team members. There are also models applicable to TAZ & BTAZ classes[5] as well as to PBDE [6]. The QSPR-THESAURUS database also contains models for other aquatic species, i.e. for *T. pyriformis*.[7]

The user can also specify data for other species by either selecting new models or/and introducing the values and their predicted or measurement errors (if available) on the web form.

In addition to the toxicity values, the user is also requested to provide the number of Monte Carlo simulations, the percentile (use 5 as default) and the confidence level in the hazardous concentration (use 50% as default). The confidence level is needed since several sources of uncertainty acts on the derived hazardous concentration: uncertainty in the SSD-model, which give rise to uncertainty in the hazardous concentration and uncertainty in the SSD data. The output presents the uncertainty that comes from uncertainty in the SSD data for a given confidence level of the hazardous concentration. Here the SSD model is a Gaussian, which gives rise to uncertainty in the hazardous concentration that depend on the uncertainty in the SSD-model modeled by a non-central Student-t distribution.

Once the required information is provided, the user can submit the calculations to the server. The server can run on any Linux or Windows machine, which has the R-language software installed.

Results of Hazardous concentration assessment based on the SSD approach are shown in Figure 10.

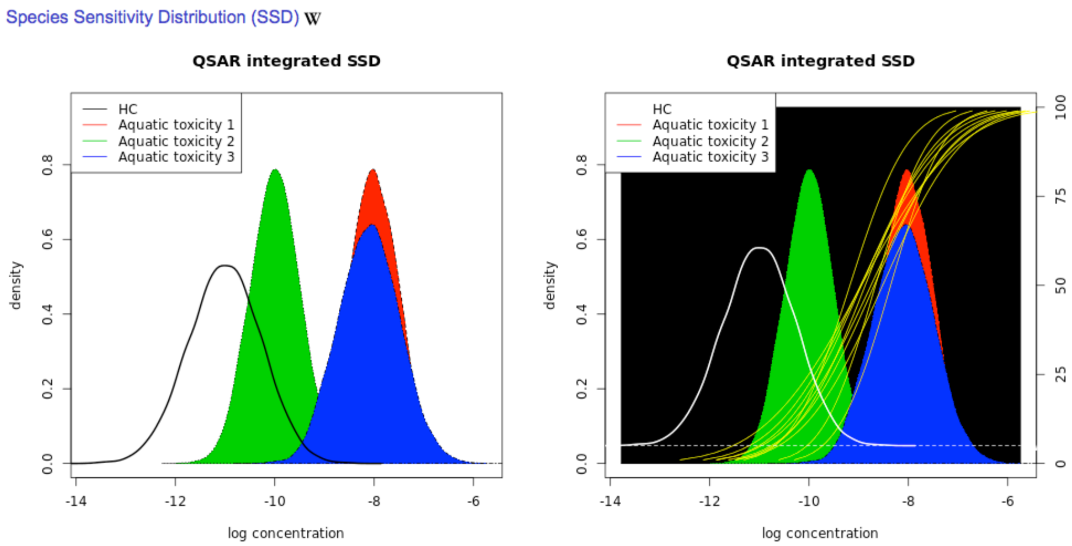


Figure 10. Uncertainty in Hazardous Concentration (HC) derived by Species sensitivity distribution approach for 2,2',3,4,4',5',6-heptabromodiphenyl ether using toxicity values computed with QSAR models developed in ref. [6]. The left plot shows SSD data for three different species with uncertainty (filled probability density functions) and the resulting density function for the uncertainty in the Hazardous Concentration. In the right plot samples from the SSD model are shown (yellow lines) that give rise to samples of the uncertainty in the HC (where the yellow lines cross the white dashed line) for the chosen level of confidence.

Like with the fate assessment, all intermediate results of calculations can be downloaded as an Excel file using the “download” button available on the page.

The distribution of values provided for the fate assessment (PEC) as well as the distribution of Hazardous Concentration upon which PNEC values can be derived using the SSD approach feed into the risk assessment. Risk, which is defined as the probability of PEC exceeding PNEC, is calculated as the integral of overlap of the respective probability density distributions. Figure 11 demonstrates probability density distributions.

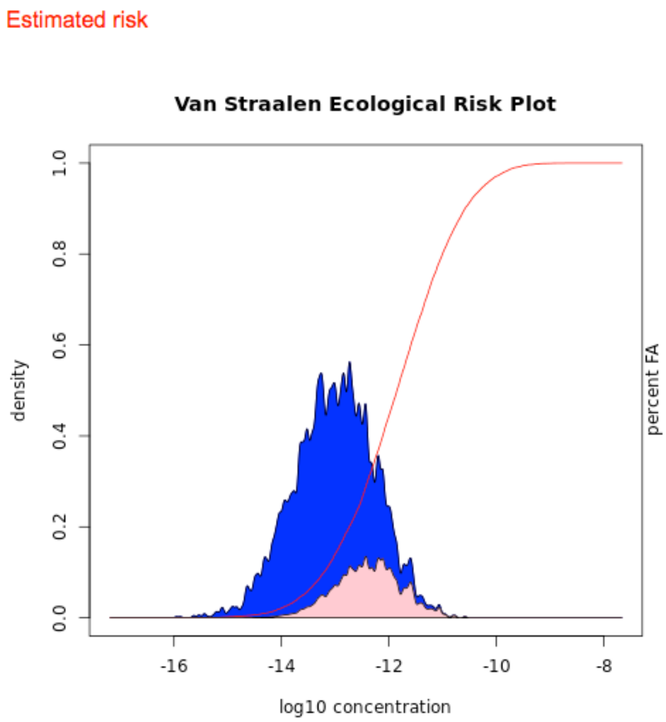


Figure 11. The estimation of the risk for PBDE-183. For this particular analysis we used 1% percentile for SSD calculations. The lower part of the right panel provides Van Straalen Ecological Risk plot,[8] which integrates both fate and effect assessment distribution. An overlap of the both distributions indicates that under the considered emission scenario this chemical could be dangerous for the environment.

The application of the developed tools to the risk assessment of all PBDEs is under preparation.[9]

Summary

We have developed a Webtool to exemplify the use of QSAR models and their predictions for the risk assessment o chemical compounds. It integrates models developed within the CADASTER project with SimpleBox[3] tools developed by RIVM scientists. The Webtool allows am easy interactive analysis for the assessment of fate, effect and risk of chemical compounds.

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Appendix 11

Prioritization of PBDEs using the QSPR-THESAURUS webtool

A prioritization of chemical compounds is important for identification of those chemicals representing the highest threat to the environment. As part of CADASTER we have developed an on-line webtool, which allows calculation of the risk of chemical compounds from the web interface. The fate assessment is provided using SimpleBox software while the toxicity estimations are based on the Species Sensitivity Distribution approach. The main purpose of this webtool is to exemplify the use of QSAR models and influence of uncertainty in the calculated physico-chemical and toxicity values on the probabilistic risk assessment of compounds. As an example, the prioritization of 207 PBDE was analyzed in details.

Introduction

The estimation of the risk of chemical compounds is the ultimate goal for the risk assessment of the molecules within REACH. The estimation of the risk of chemical compounds depends on their toxicity, emission scenario as well as physico-chemical properties, which govern their distribution, metabolism and degradation in the environment. In general the evaluation of risk is a very complex problem since chemical compounds, depending on their physico-chemical properties, could impact the environment in very different ways, e.g. some compounds can be very persistent and cause their effects over tens and even thousands of years. An example of such compounds are PFC, which have half-time degradation in atmosphere thousand years.

Several multimedia models, such as the SimpleBox tool developed in RIVM have been recently developed to provide a possibility to simulate accumulation and degradation of chemicals in different media based on a number of explicit mathematical models for transfer and degradation of molecules.

In this study, the estimation of the PEC and PNEC values was performed using SimpleBox[1] software developed by RIVM as well as the Species Sensitivity Distribution (SSD) approach.[2] Both these methods require as input physico-chemical properties of the molecules as well as data on their ecotoxicity. Since such data are usually not readily available, the risk assessors frequently face a problem either to request measurements of these parameters or/and accept the predicted values of these parameters based on the properties of similar molecules.

The QSAR methods can perform generalization of data and can be used to substitute the experimental measurements thus decreasing costs, time and in case of animals studies, also animal lives. Like experimental measurements, the QSAR predictions have uncertainties in the evaluated values. The purpose of this study was to demonstrate how one can perform risk estimation and prioritization of chemical compounds using QSAR predictions and how the uncertainty of QSAR predictions can be included for the risk estimation.

Methods

The use of SimpleBox in our study required a number of physico-chemical properties of molecules, as listed in Table 1.

Property name	Abbreviation	Units	QSAR model
Gas phase DIFFUSION coefficient	DIFFgas	[$m^2.s^{-1}$]	
Water phase DIFFUSION coefficient	DIFFwater	[$m^2.s^{-1}$]	
MOLECULAR WEIGHT	Molweight	[$g.mol^{-1}$]	
Soil Organic Carbon-Water Partitioning Coefficient	Koc	[-]	Y
Solids/water PARTITION COEFFICIENT for standard solids	Kp	[-]	
Octanol/water PARTITION COEFFICIENT	Kow	[-]	Y
Standard mass FRACTION organic carbon in soil/sediment	CORG	[-]	
Mineral DENSITY sediment and soil	RHOsolid	[$kg.m^{-3}$]	
Gas/water PARTITION COEFFICIENT at 25 °C	Kh	[-]	
VAPOR PRESSURE at 25 °C	Pvap25	[Pa]	Y
ENTHALPY of vaporization	H0vap	[$kJ.mol^{-1}$]	
Water SOLUBILITY at 25 °C	Sol25	[$mg.L^{-1}$]	Y
ENTHALPY of dissolution	H0sol	[$kJ.mol^{-1}$]	
Junge's constant	JungeConst	[Pa.m]	
Melting point	Tm	[°C]	Y
Gas phase degradation RATE CONSTANT at 25 °C	kdeg.air	[s^{-1}]	Y[a]
OH radical CONCENTRATION	C.OHrad	[cm^{-3}]	
FREQUENCY FACTOR OH radical reaction	k0.OHrad	[$cm^3.s^{-1}$]	Y
ACTIVATION ENERGY OH radical reaction	Ea.OHrad	[kJ.mol-1]	
Dissolved phase degradation RATE CONSTANT at 25 °C	kdeg.water	[s^{-1}]	Y
Biodegradability test result	biodeg	[r / r- / i / p]	
CONCENTRATION BACTERIA in test water	BACT.test	[$CFU.mL^{-1}$]	
RATE INCREASE factor per 10 °C	Q.10	[-]	
Bulk degradation RATE CONSTANT standard sediment at 25 °C	kdeg.sed	[s^{-1}]	Y[b]
Bulk degradation RATE CONSTANT standard soil at 25 °C	kdeg.soil	[s^{-1}]	Y[b]

Table 1. List of physico-chemical and biological properties required for risk assessment in SimpleBox. The Excel file, which also includes default values and formulas for calculation of some properties is provided as Supplementary Materials. [a]kdeg.air was considered to be a sum of photolytic and –OH degradation. [b]The degradation in soil and sediments were considered to be 2 and 9 times higher than in water as they are used by the USA Environmental Protection Agency (http://www.epa.gov/pbt/tools/toolbox.htm) and also in our previous study.[3]

In case experimental or predicted values are not available, the user can use pre-defined default values, which are usually selected to provide a conservative estimation of the fate assessment. Some of these parameters, such as "Standard mass FRACTION organic carbon in soil/sediment", "Gas phase DIFFUSION coefficient", "Water phase DIFFUSION coefficient", "Mineral DENSITY sediment and soil" are likely to be the same for different compounds. Several other parameters can be calculated using pre-defined formulae from other physico-chemical parameters, e.g. "Gas/water PARTITION COEFFICIENT at 25 °C" is approximated based on solubility in water and vapor pressure of chemical compounds. Of course, the user can also provide experimental values for all parameters and have more accurate estimations.

However, in the absence of both experimental and predicted values, the use of the default or calculated by formula values is the only possible solution.

In our previous study[3], QSAR models for seven physico-chemical parameters were used (see Table 1). In addition to these properties we also used QSAR model for octanol-water partition coefficient, which was developed by CADASTER project participants.[4] All these models were evaluated with respect to the possibility of their implementation in the webtool. The models for melting point (Tm, °C)[4] and vapor pressure (Vp, log(1./Pa))[4], photolysis lifetime in air ($\log(\tau_{photo\text{-}degradation})$, hour)[5] were developed using 2D descriptors and thus were easily reproduced. The model for water solubility (S, log(mol/l)) used in the previous study was based only on n=12 molecules[4] and it was not validated. Instead of this model we predicted solubility with the ALOGPS 2.1 program,[6-7] which has demonstrated very good accuracy in several studies. We have recently further developed this program to ALOGPS 3.01 by extending its training set to 8102 compounds. The ALOGPS 3.01 prediction accuracy for the PBDE molecules was RMSE=0.48, which is lower than RMSE=0.69 calculated for the whole training set. ALOGPS 3.01 thus provided a good accuracy for the PBDE compounds and can be used to model this property.

Several other models used in previous studies were based on 3D descriptors, which were calculated using Dragon as well as MOPAC programs. The chemical structures were optimized using AM1 semi-empirical method in the HYPERCHEM program (Ver. 7.03 for Windows, 2002) and descriptors were calculated using *.hin files. An attempt to re-implement them within QSPR-Thesaurus did not succeed presumably due to differences in the structure optimization protocols. Indeed, QSPR-Thesaurus uses CORINA[8] for generation of 3D structures which are further calculated using AM1 method provided by MOPAC 7.1 developed by J. Stewart.[9] These models were therefore recalculated using the standard protocol developed by the HMGU group. We used the neural network method and applied it to two sets of descriptors: E-state indices[10] and predicted solubility and octanol/water partition coefficients predicted using the ALOGPS 2.1 program[7, 11] for all analyzed properties. The performances of the developed models were evaluated using 5-fold cross-validation protocol. All newly developed models calculated similar accuracies to the previously reported models.

The biodegradation half-life time in water was estimated in the previous study according to Aronson et al.[12] Aronson mapped BIOWIN3 category predictions (from EPI Suite) to a set of experimental half-life times, which were observed for each category. The categories were formed according to the BIOWIN3 quantitative predictions (BIOWIN3 "primary" predictions). The accuracy of the model was not very high. RMSE=0.76 was calculated when predicting log(1/s) degradation times of N=249 molecules used in a Molcode model Q8-10-30-265 available at the JRC web site (http://qsardb.jrc.it). The Molcode model had RMSE=0.42 log units calculated for the training set of N=166 chemical compounds. Unfortunately, the Molcode model was developed using 3D descriptors calculated using CODESSA program,[13] which were absent in QSPR-Thesaurus database and thus could not be reproduced.

The use of the Biowin3 primary prediction to develop a regression model against all molecules produced the following model:

$$Y = 6.81 - 1.21 * \text{Biowin(primary)}, \quad RMSE=0.53, \quad N=249 \qquad (1)$$

The use of our standard neural network based approach, yielded a model with RMSE=0.4 using 5-fold cross-validation protocol for the same set of molecules. The use of the same training/set protocol as in Molcode model provided a calculated coefficient of determination of Q^2=0.86 for the same N=83 test set compounds. This coefficient was higher than Q^2=0.82 reported for the same compounds by the Molcode model authors. Thus, the newly implemented model provided the highest accuracy of predictions compared to the previous studies and it was used to estimate the degradation of chemical compounds in water.

All the developed models are accessible on the QSPR-Thesaurus database web page http://qspr-thesaurus.eu/risk.

Emission scenario

Like in the previous work, the emission of compounds to air scenario was considered. The emission of 1 tons of the compound to the air with uncertainty of 10% per year was used.

Species Sensitivity Distribution (SSD)

SSD was used to estimate the probability distribution of PEC values. The theoretical background of SSD has been explained elsewhere.[2] For the purposes of this analysis we used the 5% percentile at the 50% confidence value. The toxicity models for algae, daphnia and fish, which were described in our previous report[14] were used to predict toxicity of chemical compounds against aqua species. These three models were used in SSD calculations.

Uncertainty in input parameters

The uncertainties of the predictions were assumed to be all generated by the Gaussian distribution. While the researchers often use *t*-student distribution for linear models, its basic assumptions about identical, independent, and normally distributed models errors, are frequently not valid. In addition, the correlations of input variables and, moreover, process of variable selection cannot be easily accounted and could result in erroneous estimation of standard errors of predictions (SEP) for the predictions. The standard error of predictions is estimated as

$$[SEP(Y_p)]^2 = s^2 (1+X^t p(X^t X)^{-1} Xp)=s^2(1+h) \qquad (2)$$

where s^2 is the model error and h is leverage of the model. Indeed, as it was shown in our previous benchmarking study of several distances to models, the leverage[15] provided one of the lowest performances for the estimation of the prediction error for molecules from an independent test set, which did participate in the model development. Moreover, on the practice the h values are usually <<1 and thus the prediction errors are dominated by the average model errors. Therefore, for all studies we assumed that all predictions errors for linear models had a normal distribution with $s=s$ of the linear models.

For neural network models we estimated prediction errors based on the standard deviation of ensemble predictions.[15] This approach provided the best estimation in benchmarking studies both for regression and classification models.[15-16]

Risk estimation

After calculation of the distribution of predicted values for fate and effect assessment, one could easily estimate the risk by numerical integration of both distributions. However, since the number of steps in Monte Carlo simulations was limited to N=10,000, the estimation of risks with p < 1/N was not possible. In order to do it, we also calculated median values for both distribution and used their difference to rank molecules, which have a small risk.

Software availability

The developed software is available on http://qspr-thesaurus.eu/risk web page or as menu item "Risk Assessment" of the database. The QSPR Thesaurus web site itself was developed as an extension of On-Line CHEmical Modeling environment (OCHEM).[17] Any modern web browser, which supports Java script, can be used to run it.

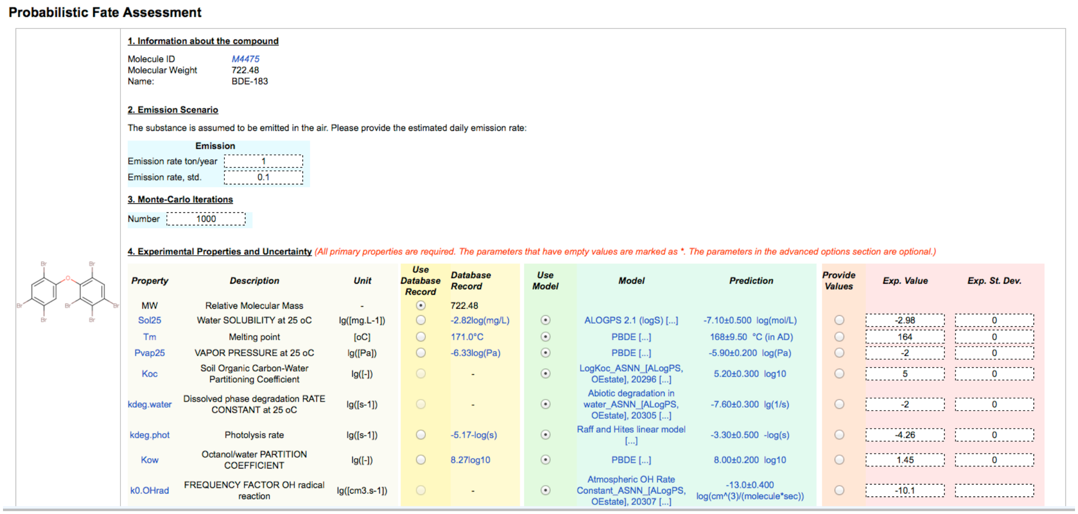


Figure 1. Graphical interface to introduce properties of a molecule for the fate assessment. The user can use values stored in the database (white area), calculate properties using models (blue area) and also provide them (red area) as expert knowledge.

Figure 1 demonstrates the graphical interface to specify data for the fate assessments. The user can select either experimental values, which are available in the QSPR-database, use model predictions or provide his/her values for some of parameters based on his expert knowledge. All intermediate results from the Monte Carlo simulation runs both for fate and effect assessments can be downloaded and used for the off-line analysis.

Results and discussions

Table 2 indicates the estimate risk estimations for all 207 compounds from the PBDE dataset.

Table 2. Predicted risks for PBDEs.

N	NAME	Number of Br	Predicted Risk
1	2-monoBDE	1	0.0%
2	3-monoBDE	1	0.0%
3	4-monoBDE	1	0.0%
4	2,2'-diBDE	2	0.0%
5	2,3-diBDE	2	0.0%
6	2,3'-diBDE	2	0.0%
7	2,4-diBDE	2	0.0%
8	2,4'-diBDE	2	0.0%
9	2,5-diBDE	2	0.0%
10	2,6-diBDE	2	0.0%
11	3,3'-biBDE	2	0.0%
12	3,4-diBDE	2	0.0%
13	3,4'-diBDE	2	0.0%
14	3,5-diBDE	2	0.0%
15	4,4'-diBDE	2	0.0%
16	2,2',3-triBDE	3	0.0%
17	2,2',4-triBDE	3	0.0%
18	2,2',5-triBDE	3	0.0%
19	2,2',6-triBDE	3	0.0%
20	2,3,3'-triBDE	3	0.0%
21	2,3,4-triBDE	3	0.0%
22	2,3,4'-triBDE	3	0.0%
23	2,3,5-triBDE	3	0.0%
24	2,3,6-triBDE	3	0.0%
25	2,3',4-triBDE	3	0.0%
26	2,3',5-triBDE	3	0.0%
27	2,3',6-triBDE	3	0.0%
28	2,4,4'-triBDE	3	0.0%
29	2,4,5-triBDE	3	0.0%
30	2,4,6-triBDE	3	0.0%
31	2,4',5-triBDE	3	0.0%
32	2,4',6-triBDE	3	0.0%
33	2,3',4'-triBDE	3	0.0%
34	2,3',5'-triBDE	3	0.0%
35	3,3',4-triBDE	3	0.0%
36	3,4,4'-triBDE	3	0.0%

37	3,4,5-triBDE	3	0.0%
38	3,4',5-triBDE	3	0.0%
39	2,2',3,3'-tetraBDE	4	0.0%
40	2,2',3,4-tetraBDE	4	0.0%
41	2,2',3,4'-tetraBDE	4	0.0%
42	2,2',3,5-tetraBDE	4	0.0%
43	2,2',3,5'-tetraBDE	4	0.0%
44	2,2',3,6-tetraBDE	4	0.0%
45	2,2',3,6'-tetraBDE	4	0.0%
46	2,2',4,4'-tetraBDE	4	0.2%
47	2,2',4,5-tetraBDE	4	0.0%
48	2,2',4,5'-tetraBDE	4	0.0%
49	2,2',4,6-tetraBDE	4	0.0%
50	2,2',4,6'-tetraBDE	4	0.0%
51	2,2',5,5'-tetraBDE	4	0.0%
52	2,2',5,6'-tetraBDE	4	0.0%
53	2,2',6,6'-tetraBDE	4	0.0%
54	2,3,3',4-tetraBDE	4	0.0%
55	2,3,3',4'-tetraBDE	4	0.0%
56	2,3,3',5-tetraBDE	4	0.0%
57	2,3,3',5'-tetraBDE	4	0.0%
58	2,3,3',6-tetraBDE	4	0.0%
59	2,3,4,4'-tetraBDE	4	0.2%
60	2,3,4,5-tetraBDE	4	0.0%
61	2,3,4,6-tetraBDE	4	0.0%
62	2,3,4',5-tetraBDE	4	0.0%
63	2,3,4',6-tetraBDE	4	0.0%
64	2,3,5,6-tetraBDE	4	0.0%
65	2,3',4,4'-tetraBDE	4	0.0%
66	2,3',4,5-tetraBDE	4	0.0%
67	2,3',4,5'-tetraBDE	4	0.1%
68	2,3',4,6-tetraBDE	4	0.0%
69	2,3',4',5-tetraBDE	4	0.0%
70	2,3',4',6-tetraBDE	4	0.0%
71	2,3',5,5'-tetraBDE	4	0.0%
72	2,3',5',6-tetraBDE	4	0.0%
73	2,4,4',5-tetraBDE	4	0.0%
74	2,4,4',6-tetraBDE	4	0.0%
75	2,3',4',5'-tetraBDE	4	0.0%
76	3,3',4,4'-tetraBDE	4	1.1%
77	3,3',4,5-tetraBDE	4	0.7%
78	3,3',4,5'-tetraBDE	4	0.3%
79	3,3',5,5'-tetraBDE	4	0.1%

80	3,4,4',5-tetraBDE	4	0.8%
81	2,2',3,3',4-pentaBDE	5	0.4%
82	2,2',3,3',5-pentaBDE	5	0.4%
83	2,2',3,3',6-pentaBDE	5	0.0%
84	2,2',3,4,4'-pentaBDE	5	3.4%
85	2,2',3,4,5-pentaBDE	5	0.5%
86	2,2',3,4,5'-pentaBDE	5	0.9%
87	2,2',3,4,6-pentaBDE	5	0.0%
88	2,2',3,4,6'-pentaBDE	5	0.0%
89	2,2',3,4',5-pentaBDE	5	3.4%
90	2,2',3,4',6-pentaBDE	5	0.1%
91	2,2',3,5,5'-pentaBDE	5	1.5%
92	2,2',3,5,6-pentaBDE	5	0.0%
93	2,2',3,5,6'-pentaBDE	5	0.0%
94	2,2',3,5',6-pentaBDE	5	0.0%
95	2,2',3,6,6'-pentaBDE	5	0.0%
96	2,2',3,4',5'-pentaBDE	5	1.2%
97	2,2',3,4',6'-pentaBDE	5	0.6%
98	2,2',4,4',5-pentaBDE	5	5.1%
99	2,2',4,5,5'-pentaBDE	5	2.7%
100	2,2',4,5,6'-pentaBDE	5	0.4%
101	2,2',4,5',6-pentaBDE	5	0.7%
102	2,2',4,6,6'-pentaBDE	5	0.3%
103	2,3,3',4,4'-pentaBDE	5	5.7%
104	2,3,3',4,5-pentaBDE	5	2.7%
105	2,3,3',4',5-pentaBDE	5	4.7%
106	2,3,3',4,5'-pentaBDE	5	5.4%
107	2,3,3',4,6-pentaBDE	5	1.6%
108	2,3,3',4',6-pentaBDE	5	1.6%
109	2,3,3',5,5'-pentaBDE	5	5.0%
110	2,3,3',5,6-pentaBDE	5	0.3%
111	2,3,3',5',6-pentaBDE	5	0.7%
112	2,3,4,4',5-pentaBDE	5	5.2%
113	2,3,4,4',6-pentaBDE	5	3.2%
114	2,3,4,5,6-pentaBDE	5	0.0%
115	2,3,4',5,6-pentaBDE	5	1.8%
116	2,3',4,4',5-pentaBDE	5	8.3%
117	2,3',4,4',6-pentaBDE	5	5.6%
118	2,3',4,5,5'-pentaBDE	5	6.6%
119	2,3',4,5',6-pentaBDE	5	5.9%
120	2,3,3',4',5'-pentaBDE	5	2.6%
121	2,3',4,4',5'-pentaBDE	5	7.9%
122	2,3',4',5,5'-pentaBDE	5	5.8%

123	2,3',4',5',6-pentaBDE	5	1.6%
124	3,3',4,4',5-pentaBDE	5	9.9%
125	3,3',4,5,5'-pentaBDE	5	10.2%
126	2,2',3,3',4,4'-hexaBDE	6	10.4%
127	2,2',3,3',4,5-hexaBDE	6	7.0%
128	2,2',3,3',4,5'-hexaBDE	6	12.3%
129	2,2',3,3',4,6-hexaBDE	6	3.9%
130	2,2',3,3',4,6'-hexaBDE	6	4.2%
131	2,2',3,3',5,5'-hexaBDE	6	15.3%
132	2,2',3,3',5,6-hexaBDE	6	1.5%
133	2,2',3,3',5,6'-hexaBDE	6	5.1%
134	2,2',3,3',6,6'-hexaBDE	6	0.4%
135	2,2',3,4,4',5-hexaBDE	6	15.4%
136	2,2',3,4,4',5'-hexaBDE	6	18.3%
137	2,2',3,4,4',6-hexaBDE	6	12.2%
138	2,2',3,4,4',6'-hexaBDE	6	13.2%
139	2,2',3,4,5,5'-hexaBDE	6	11.5%
140	2,2',3,4,5,6-hexaBDE	6	1.3%
141	2,2',3,4,5,6'-hexaBDE	6	3.5%
142	2,2',3,4,5',6-hexaBDE	6	6.9%
143	2,2',3,4,6,6'-hexaBDE	6	1.7%
144	2,2',3,4',5,5'-hexaBDE	6	19.7%
145	2,2',3,4',5,6-hexaBDE	6	7.9%
146	2,2',3,4',5,6'-hexaBDE	6	15.5%
147	2,2',3,4',5',6-hexaBDE	6	7.5%
148	2,2',3,4',6,6'-hexaBDE	6	6.0%
149	2,2',3,5,5',6-hexaBDE	6	5.2%
150	2,2',3,5,6,6'-hexaBDE	6	0.7%
151	2,2',4,4',6,6'-hexaBDE	6	16.8%
152	2,3,3',4,4',5-hexaBDE	6	23.2%
153	2,3,3',4,4',5'-hexaBDE	6	23.7%
154	2,3,3',4,4',6-hexaBDE	6	16.9%
155	2,3,3',4,5,5'-hexaBDE	6	25.2%
156	2,3,3',4,5,6-hexaBDE	6	6.4%
157	2,3,3',4,5',6-hexaBDE	6	19.1%
158	2,3,3',4',5,5'-hexaBDE	6	27.1%
159	2,3,3',4',5,6-hexaBDE	6	12.6%
160	2,3,3',4',5',6-hexaBDE	6	11.8%
161	2,3,3',5,5',6-hexaBDE	6	15.4%
162	2,3,4,4',5,6-hexaBDE	6	9.5%
163	2,3',4,4',5,5'-hexaBDE	6	31.3%
164	2,3',4,4',5',6-hexaBDE	6	26.6%
165	3,3',4,4',5,5'-hexaBDE	6	46.2%

166	2,2',3,3',4,4',5-heptaBDE	7	32.4%
167	2,2',3,3',4,4',6-heptaBDE	7	25.1%
168	2,2',3,3',4,5,5'-heptaBDE	7	32.3%
169	2,2',3,3',4,5,6-heptaBDE	7	13.4%
170	2,2',3,3',4,5,6'-heptaBDE	7	19.1%
171	2,2',3,3',4,5',6-heptaBDE	7	25.7%
172	2,2',3,3',4,6,6'-heptaBDE	7	12.3%
173	2,2',3,3',4,5',6'-heptaBDE	7	17.9%
174	2,2',3,3',5,5',6-heptaBDE	7	17.2%
175	2,2',3,3',5,6,6'-heptaBDE	7	7.3%
176	2,2',3,4,4',5,5'-heptaBDE	7	40.3%
177	2,2',3,4,4',5,6-heptaBDE	7	25.2%
178	2,2',3,4,4',5,6'-heptaBDE	7	35.0%
179	2,2',3,4,4',6,6'-heptaBDE	7	25.8%
180	2,2',3,4,5,5',6-heptaBDE	7	18.7%
181	2,2',3,4,5,6,6'-heptaBDE	7	7.2%
182	2,2',3,4',5,5',6-heptaBDE	7	24.7%
183	2,2',3,4',5,6,6'-heptaBDE	7	17.8%
184	2,3,3',4,4',5,5'-heptaBDE	7	53.0%
185	2,3,3',4,4',5,6-heptaBDE	7	31.3%
186	2,3,3',4,4',5',6-heptaBDE	7	39.4%
187	2,3,3',4,5,5',6-heptaBDE	7	33.8%
188	2,3,3',4',5,5',6-heptaBDE	7	34.2%
189	2,2',3,3',4,4',5,5'-octaBDE	8	100.0%
190	2,2',3,3',4,4',5,6-octaBDE	8	75.3%
191	2,2',3,3',4,4',5,6'-octaBDE	8	78.2%
192	2,2',3,3',4,4',6,6'-octaBDE	8	77.1%
193	2,2',3,3',4,5,5',6-octaBDE	8	72.0%
194	2,2',3,3',4,5,5',6'-octaBDE	8	74.2%
195	2,2',3,3',4,5,6,6'-octaBDE	8	52.3%
196	2,2',3,3',4,5',6,6'-octaBDE	8	65.3%
197	2,2',3,3',5,5',6,6'-octaBDE	8	52.9%
198	2,2',3,4,4',5,5',6-octaBDE	8	81.8%
199	2,2',3,4,4',5,6,6'-octaBDE	8	72.7%
200	2,3,3',4,4',5,5',6-octaBDE	8	100.0%
201	2,2',3,3',4,4',5,5',6-nonaBDE	9	100.0%
202	2,2',3,3',4,4',5,6,6'-nonaBDE	9	100.0%
203	2,2',3,3',4,5,5',6,6'-nonaBDE	9	100.0%
204	2,2',3,3',4,4',5,5',6,6'-decaBDE	10	100.0%
205	4'-OH-2,4,6-tribromodiphenyl ether	3	22.8%
206	4'-OH-2,3',4,6-tetrabromodiphenyl ether	4	40.3%
207	4'-OH-2,3',4,5',6-pentabromodiphenyl ether	5	100.0%

The graphical representation of the calculated results is shown in Figure 2.

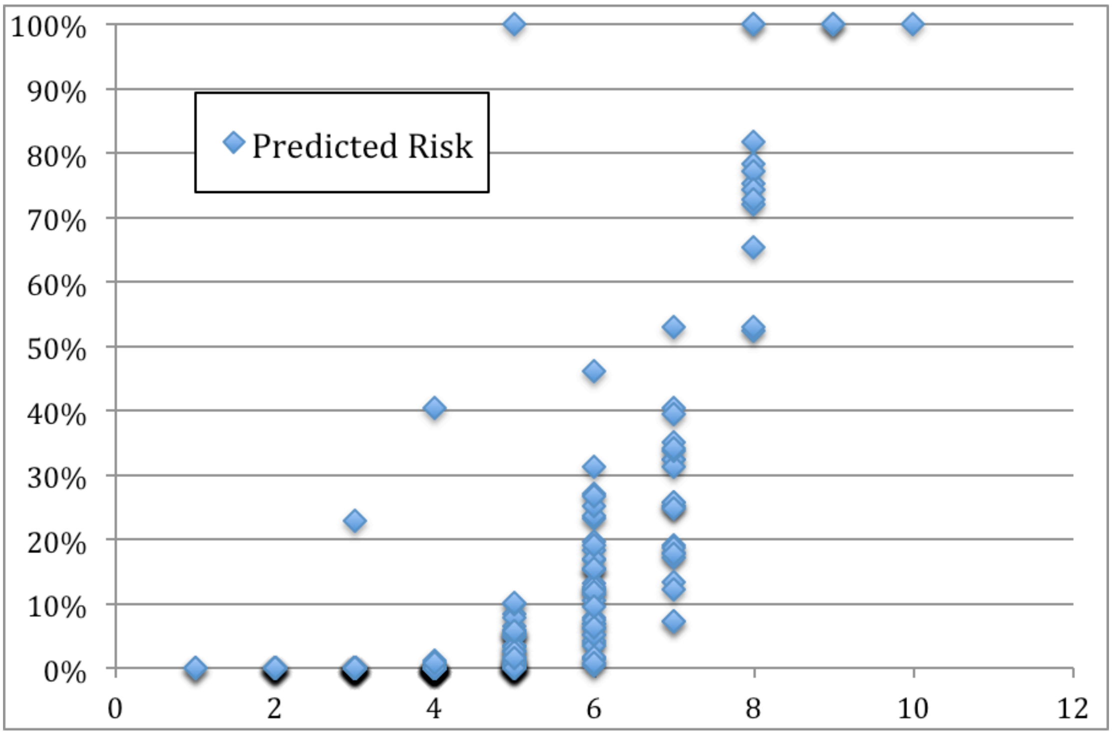
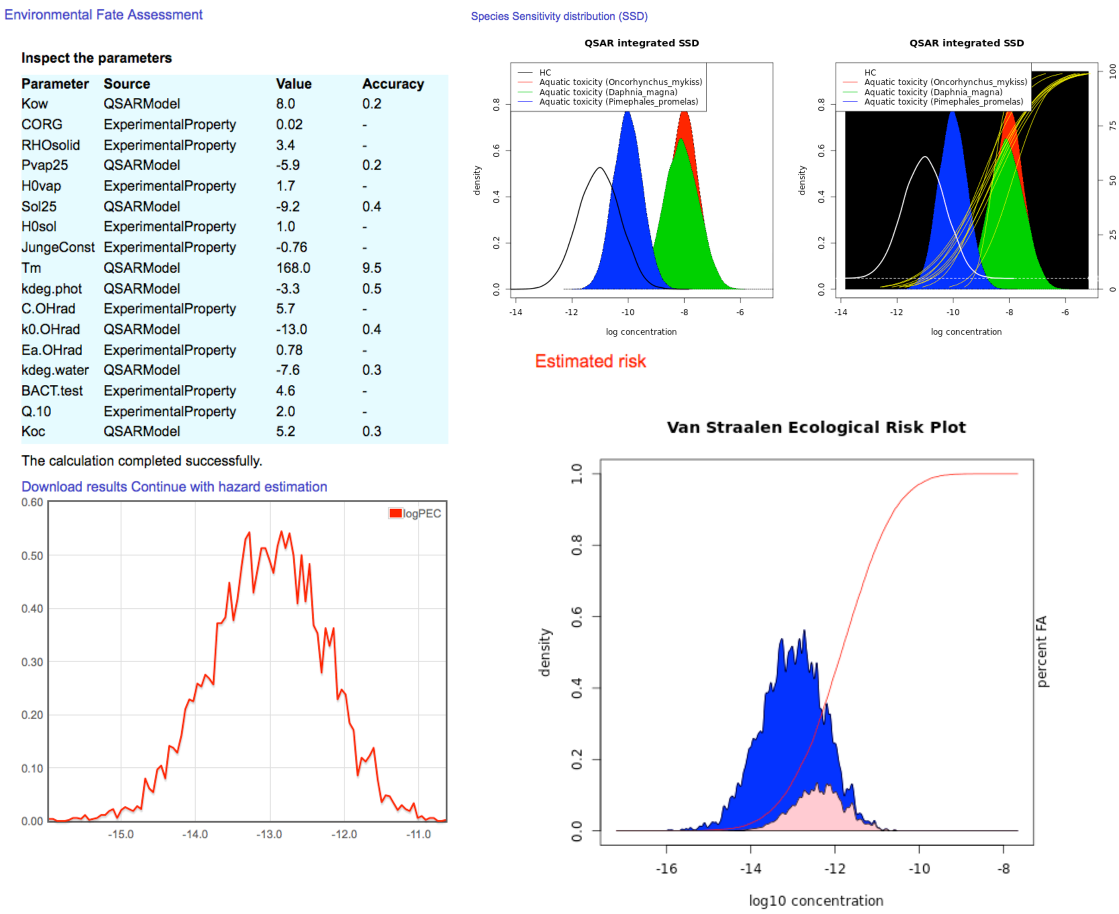


Figure 2. Environmental risk for PBDE as function of the number of Br atoms in the molecule.

It is clear that compounds with large number of Br represent a significant risk to the environment. Indeed, all PBDE with more than 5 atoms have predicted non-zero risk and could be toxic to the aquatic species under the considered emission scenario. At the same time molecules with 1-3 atoms are not predicted as environmental pollutants. It is interesting that the model also predicts toxicity for three ethers, namely 4'-OH-2,4,6-tribromodiphenyl ether and 4'-OH-2,3',4,6-tetrabromodiphenyl ether which have only 3, and 4 Br atoms, respectively. Both these molecules are phenols. Another phenol, 4'-OH-2,3',4,5',6-pentabromodiphenyl ether, which has 5 atoms, is also more toxic (predicted risk is 100%) that other PBDE with the same number of atoms.

An example of a risk assessment for PBDE-183 is indicated in Figure 3.



Parameter	Source	Value	Accuracy
Kow	QSARModel	8.0	0.2
CORG	ExperimentalProperty	0.02	-
RHOsolid	ExperimentalProperty	3.4	-
Pvap25	QSARModel	-5.9	0.2
H0vap	ExperimentalProperty	1.7	-
Sol25	QSARModel	-9.2	0.4
H0sol	ExperimentalProperty	1.0	-
JungeConst	ExperimentalProperty	-0.76	-
Tm	QSARModel	168.0	9.5
kdeg.phot	QSARModel	-3.3	0.5
C.OHrad	ExperimentalProperty	5.7	-
k0.OHrad	QSARModel	-13.0	0.4
Ea.OHrad	ExperimentalProperty	0.78	-
kdeg.water	QSARModel	-7.6	0.3
BACT.test	ExperimentalProperty	4.6	-
Q.10	ExperimentalProperty	2.0	-
Koc	QSARModel	5.2	0.3

Figure 3. The estimation of the risk for PBDE-183. The left panel indicates properties of molecules and predicted fate distribution for the analyzed compound. The upper part of the right panel indicates effect assessment, which is calculated using SSD. For this particular analysis we used 1% percentile for SSD calculations. The lower part of the right panel provides Van Straalen Ecological Risk plot,[18] which integrates both fate and effect assessment distribution. An overlap of the both distributions indicates that under the considered emission scenario this chemical could be dangerous for the environment.

The developed web-tool also allows visually inspection of the impact of individual physico-chemical parameters on the fate assessment of chemical compounds via sensitivity analysis. The sensitivity is calculated as Spearman Rank correlations between each individual parameter and the calculated fate effect. The use of Monte Carlo simulations thus allows identifying which parameters have the highest impact on the resulting concentration of the chemicals in the environment.

Figure 4 indicates that for the fate assessment of PBDE-183 the highest impact is provided by the model to predict degradation in air via photolysis. If this mechanism is not considered the degradation in air due to hydroxyl radical reaction rate, which is about 5 folds smaller to that of photolysis, does not provide a major effect on the accumulation of chemicals in water. In the latter case the dominating effects become solubility of the molecule. It indicates non-linear effects of the different parameters for the fate assessment of the molecules.

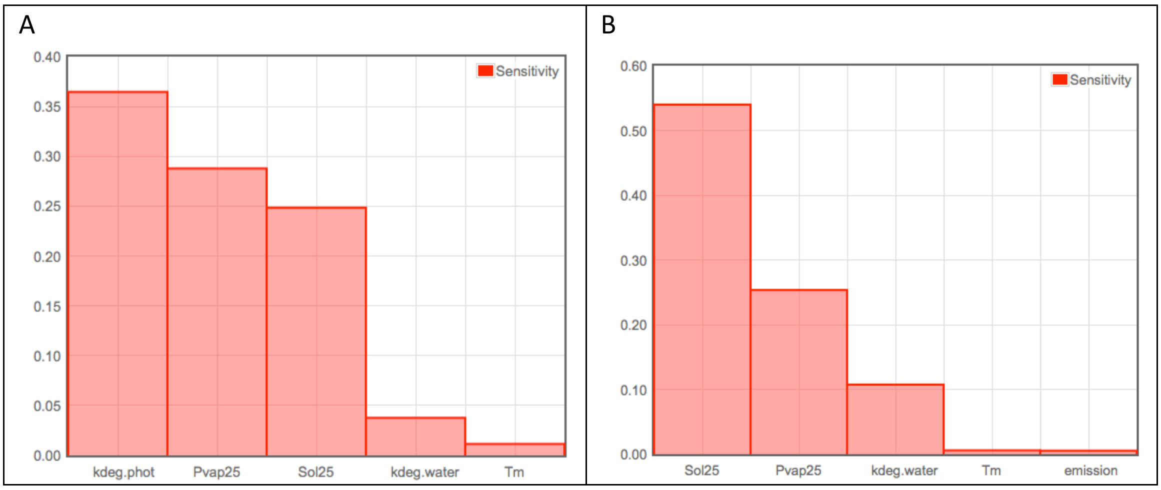


Figure 4. Sensitivity analysis of the most important properties determining the fate of PBDE-183. Panel A: In case if photolysis in air is considered ($k_{deg.phot}$), the variability of this model provides the highest impact on the fate of this chemical compound. Panel B: in case if photolysis is not taken into the account, solubility in water becomes the most influential parameter for the accumulation of this compound in water.

Conclusions

We have developed a web tool for the exemplification of the use of QSAR models for the fate, effect and risk assessment of chemical compounds. The developed webtool allows an easy and interactive analysis of the fate and effect assessment of chemical compounds using Simple Box and the SSD approach, respectively. The PEC and PNEC values calculated with both these approaches can be used to provide a risk assessment of chemicals. We have also demonstrated its use for the risk assessment of 207 PBDE. The use of the developed tool is limited to the availability of experimental values and QSAR models for the analyzed classes of molecules. The QSPR-Thesaurus database contains a number of such models described in multiple publications,[3-4, 19-25] which were developed during the CADASTER project for the four classes of compounds. However, in case some of the models or experimental values are not available in the database or/and in case an analysis is required for classes not yet available in the database, the user can provide the required properties as his/her expert knowledge. Thus, the developed approach can be easily used for the estimation of the risk assessment of new classes of molecules beyond those analyzed in the CADASTER project.

Acknowledgements

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