Scientific Concepts and Tools for Sustainable Chemistry as Applied to Ionic Liquids and Antifouling Biocides

Habilitationsschrift
zur Erlangung der *venia legendi*
im Fach Umweltchemie und Ökotoxikologie
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vorgelegt von
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Preface to the version available on the internet

This internet version of the thesis only differs from the version that was turned in 18 months ago in the following way: The copyrighted papers have been removed and replaced by abstracts and links to the abstracts on the web as hosted by the publishers. The water solubility paper and the R news paper are still fully included. Three papers were published after submission of the thesis, so the references were updated accordingly.

Grenzach, January 2010

Preface

The research described in this thesis was carried out in the period from October 2001 until June 2008 in the Department Bioorganic Chemistry, now Department Sustainable Chemistry, of the Centre for Environmental Research and Sustainable Technologies (UFT) of the University of Bremen

My position in this time was funded mainly by the University of Bremen, but in parts also by the Deutsche Bundesstiftung Umwelt DBU, the Federal Ministry of Education and Research bmb+f, and the Senator für Bau, Umwelt und Verkehr of the state of Bremen.

I would like to say thank you to the many people who have contributed to the work which was in fact team work in large parts. I am grateful that my former supervisor Prof. Dr. Bernd Jastorff has given me the possibility to make the research on antifouling biocides a constant in the working group, and who furthermore gave me the freedom to develop my contributions to the research on ionic liquids in the directions that I found promising and relevant.

I would like to thank all coauthors of the scientific papers that form part of this thesis. I explicitly thank the technicians, colleagues and the former lab rotation, diploma and doctoral students in the group for support and collaboration, namely Ulrike Bottin-Weber, Peter Behrend and Andrea Böschen, Dr. Frauke Stock, Dr. Caren Doose, Matthias Dünne, Dr. Christian Jungnickel, Frauke Nehen, Ping Fan, Dr.med. Alaa Othman, Salha Abdulkarim, Ioana Stan, Dr. Stefan Stolte, Marianne Matzke and Jürgen Arning. With their great team spirit and effectiveness, all of them have contributed greatly to the research of our group, and thus also to this thesis.

Special thanks go to Anja Müller, for the great collaboration and experimental support during the development of the hydrophobicity parameters for the ionic liquids and on other occasions, and to Dr. Reinhold Störmann, the long-time member and associate of the group, for his diligent work on and input to the substance databases described here, as well as for joining into the exploration and employment of Open Source tools.

The excellent work of UFT staff in the persons of Ruth Krumrey-Rosch, Antje Mathews, Dr. Kerstin Mölter and Falko Berger is highly appreciated, as well as the nice and productive working atmosphere established by all UFT colleagues.

Catalysed by the retirement of Prof. Jastorff last fall, it was a pleasure to enter into an

increasingly close colloboration in several research projects with the UFT Department of Chemical Engineering — Recovery and Recycling, headed by Prof. Dr. Jorg Thöming. Not only I am deeply indebted to him for his ability and willingness to dive into the scientific and organisational aspects of taking on large parts of the responsibility for a second working group.

The ecotoxicological parts of this work would not have been possible without the long-standing fruitful collaboration with the UFT Department of General and Theoretical Ecology, headed by Prof. Dr. Juliane Filser, who inherited the *Scenedesmus vacuolatus* reproduction assay from the well-known former working group of Prof. L. Horst Grimme.

The support of Merck KGaA within the strategic partnership in the field of ionic liquid development, especially in the persons of Dr. Urs Welz-Biermann and Dr. William-Robert Pitner is gratefully acknowledged. I feel lucky to have had the possibility to take part in this.

This is also a good opportunity to thank the authors of and contributors of Open Source software in general, and in particular of the tools that I used in this work.

I would like to thank Dr. Simon Spycher for still keeping up the ETH environmental chemistry connection (for whatever that may be), and my other friends — for their friendship.

Finally, I would like to thank the four grandparents of my children for lots of support in turbulent times, and, most importantly, my wife and my children for their patience with my sometimes excessive preoccupation with science.

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1 Introduction

Chemistry, at its core, is the science and human practice of purification, transformation and characterization of physical matter. Both aspects, science and human practice, have grown to enormous power and complexity in the short period of maybe 200 years since it started to take its current shape, losing more and more the traits of a natural history, and becoming an integral part of industrial society.[1]

The German sociologist and philosopher Niklas Luhmann proposed to distinguish three dimensions of "sense",[2] which he describes as a common accomplishment that has emerged from the co-evolution of psychic and social systems (Luhmann, 1999, p. 92ff). The three dimensions, which can also be viewed as world dimensions (*ibidem*, p. 112), are there to specify subject ("Sachdimension"), time ("Zeitdimension"), and *alter ego* ("Sozialdimension") of sense.[3]

In all three dimensions can chemistry be reductionistic to an extreme and fascinating degree. Taking part in todays diversified and disjunct system of scientific disciplines, it oftentimes restricts its subject to processes and constellations at the molecular level, to the time immediately before and immediately after a reaction, and the only *alter ego* involved is the specialized fellow chemist or a specific scientific community.

On the other hand, chemical products, the language and the concepts of chemistry have pervaded our world. The spatial range of chemicals (Scheringer, 1996) may be as large as the circumference of the earth, the deep sea, and the stratosphere. Their temporal range may extend to tens of thousands of years, *e.g.* in the sediments of the deep sea (Ranke, 2002). Individuals and societies pondering about chemical processes and products include people with various different kinds of education and most human societies. In addition to the psychic and social systems considered by Luhmann, even physiological systems like the immune system or the endocrine system of vertebrates can be considered as an *alter ego*, certainly with a sense for the chemical nature of matter.

1.1 Challenges and conditions for a more sustainable chemistry

The divergence between the powerful reductionist approach and the universal relevance of chemistry described above leads to the formulation of three challenges for the development of sustainable chemistry:

[1] All trademarks used in this thesis are the property of their respective owners.

[2] The German word "Sinn" used by Luhmann and the English word "sense" have similar meanings.

[3] The term *alter ego* refers to the viewpoint of another consciousness: "Man kann allen Sinn daraufhin abfragen, ob ein anderer ihn genau so erlebt wie ich oder anders." (For every sense, the question can be asked if somebody else experiences it in the same way as I do, or in another way, Luhmann, 1999, p. 119)

1. Chemical interactions outside the lab and outside of technical systems in general have to be considered.

2. The time scale relevant for a specific chemical substance includes its history and its residence time in the environment.

3. Actors in chemistry have to take into account possible diverging perspectives on their actions, including different educational backgrounds and cultural paradigms, and maybe even the perspective of physiological systems.

Over the years, all of these challenges have been addressed in some way or the other. Biochemistry, Toxicology and Environmental Chemistry have evolved. The history of substances[4] is generally included in Life Cycle Analysis (LCA) which has been performed for various chemical products. There is a large body of literature on the persistence of chemical substances and their degradation products, and this criterion has found its way into the international Stockholm convention on Persistent Organic Pollutants (POPs) as well as into the European REACH regulation, among many others. Many chemists, the large chemical societies and the chemical industry have recognized the need for a dialogue with different parts of society about the value and the risks of dealing with chemistry.

The task of sustainable chemistry is often understood as finding ways to help modern society to be sustainable, for example by developing more efficient catalysts. However, the above challenges imply that chemistry in itself has to strive for sustainability. In the opinion of the author there is still a lot to do with respect to complementing reductionism with a more holistic view within the chemical endeavor and a precondition for a truly sustainable chemistry is the constant evaluation of the pathways that have been chosen.

1.2 Scientific scope of sustainable chemistry

The first of the challenges listed above entails that knowledge generated by Biochemistry, Toxicology and Environmental Chemistry is vital for a serious consideration of the interactions of chemical substances with the environment and the biological organisms therein. Because of the vast amount of detailed information that is available today, it is especially important to take advantage of generalizations. This means that finding common aspects in these disciplines is especially valuable, because it helps reducing the complexity of the problem, even though this strategy is limited by the principal differences between the disciplines.

Secondly, sustainable chemistry has to take the history of the materials into account, which it deals with. This should go much further than stating the experimental details of the synthesis that was used for the last step of chemical transformation, or the commercial source. Apart from the LCA approach, the use of renewable feedstocks in chemistry

[4] Since the relevance of time scales and histories of substances has not been excessively addressed in the context of sustainable chemistry, the reader is referred to a contribution by Huppenbauer and Reller (1996).

is an attempt to respect the importance of the history of the materials we are dealing with. Chemistry also has to deal with the fate of chemical substances in time, which easily translates into the necessity to monitor them in space. While this is dealt with in the field of mathematical fate modelling, today oftentimes incorporating the reflexive element of uncertainty analyses, it has to be complemented by common sense, and a sense for the borderline between the kind of knowledge that can be generated, and what we can not know (Hoffmann-Riem and Wynne, 2002; Böschen et al., 2004).

Finally, the possibility that others judge ones doings from a different perspective, and that they could even come to the conclusion that one is completely wrong, must be taken into account. This includes recognizing the limited scope of the scientific viewpoint itself (Weinberg, 1972). Every social subsystem, including science, has its blind spots, but today we can be aware of this.

1.3 Approaches to sustainable chemistry

Several approaches have been taken to analyse and improve the relationship between chemistry and sustainable development[5]. The following treatment is not meant to be complete, but to cover the most prominent types of approaches, especially concerning chemical products.

Hermann Fischer (1993) has characterized the vision of a "gentle chemistry" in 9 theses. One of the more controversial theses is that the potential of chemical substances to do harm in the environment is proportional to the violence that is used in generating the substances. At least it can be stated that this is not generally accepted, so it is a weak basis for meeting challenge 1 from above. Challenge 2 is fully covered by the first out of the nine theses. The social dimension of dealing with chemistry (Challenge 3) is not considered. The concept of violence against physical matter can be seen as an attempt to take into account its "viewpoint" as an *alter ego*. However, a theoretical foundation for such an interpretation is lacking.

A related approach is the use of penetration depth and a related set of criteria as indicators for technology assessment, which has been proposed by Arnim von Gleich (1998, for example), which is motivated by ethical considerations and the precautionary principle, in a similar way as the criteria of spatial and temporal range (persistence) developed by Berg and Scheringer (1994, and later publications by the groups of Müller-Herold and Scheringer). Both approaches conceptually meet Challenge 1 and 2, but do not address Challenge 3.

In the United States of America, the term Green Chemistry has been branded (Anastas and Williamson, 1994; Anastas and Warner, 1998). It is characterized by ten principles, and a price for Green Chemistry is regularly awarded by the presidential office. The ten principles cover challenges 1 and 2, but they also do not address the possibility of diverging viewpoints. The high degree of operationalization with the ten principles and

[5] An excellent overview of ethical aspects and industrial approaches to sustainability has been given by Müller-Eisen and Hulpke (2000).

the evaluation practice during the selection of the award winning products makes it a pragmatic and successful approach.

Sustainability evaluation systems that have been developed in the context of the European Chemical Industry (Ewen et al., 1997; Steuerungsgruppe zum Dialogprojekt PVC und Nachhaltigkeit und Arbeitsgemeinschaft PVC und Umwelt e.V., 1999; Saling et al., 2005) are generally based on a system, rather than a list of criteria, which in turn is based on the Brundtland definition of sustainable development, asking for an equal treatment of economical, ecological, and social aspects. Therefore, these evaluation systems do incorporate society related aspects (for a rationale for this term see Böschen et al., 2004), and some of the indicators covering the social dimension of sustainable development do take into account the possibility and relevance of alternative viewpoints.

The differing approaches of Green Chemistry and Sustainable Chemistry have lead to quite a lot of debate (for an early, short account see Hutzinger, 1999). In Japan and the US, it seems that there is now consensus to use the term "Green and Sustainable Chemistry", while in Germany, recently a new working group called "Nachhaltige Chemie" (sustainable chemistry) has been established within the German Chemical Society (GDCh), that does not use the term "Green".

A personal vision for more sustainability in chemistry has been put forward by Bernd Jastorff et al. (2003b, 2007), claiming that a more efficient use of structure-activity relationships would pave the way to a more sustainable chemistry. This approach mainly focusses on challenge 1 stated above.

The European Chemical Industry has established the European Technology Platform for Sustainable Chemistry (SusChem), representing an impressive list of companies and institution, with the "fundamental goal [...] to contribute to a sustainable quality of life for all Europe's citizens" (SusChem Website). This technology platform exactly represents the approach of chemistry to help society to be sustainable. The sustainability of the chemistry employed in this process is not so much the subject of debate in this context.

Clearly, the manifold approaches to sustainable chemistry have a common motivation. However, the methods applied and the research topics derived from this motivation differ greatly. Some approaches mainly consist of sustainability evaluations, applied to chemical technologies or products. Some others mainly deal with an improvement of such technologies and products themselves, presupposing that the results will improve sustainability, without a detailed discussion of the implications the new technology will have with respect to the wide scope of sustainability.

It is the view of the author that the most effective approaches toward a more sustainable chemistry consist of both technology improvements, and at the same time of their reflective evaluations, addressing all three challenges, by widening their scope to the chemistry outside of the lab and/or technical systems, by extending the time scale under scrutiny, and by taking into account possible alternative viewpoints.

1.4 Outline of the thesis

The results described in this thesis have been achieved in three work areas: risk analysis of antifouling biocides used in paints and coatings[6] sustainable product design of ionic liquids[7], and higher education for more sustainability in chemistry. In Chapter 2, the most important concepts used in the first two areas of work are briefly described in order to provide a common theoretical basis. In Chapter 3, the tools developed by the author in the course of the period covered by this thesis are explained. In Chapter 4, the most important advances that resulted from the application of concepts and tools to the three work areas are shown.

Chapter A consists of the publications in which the main results from this work have been or are being published in peer-reviewed journals. Chapter B contains further publications, in which the author of this thesis is corresponding author, but to which the contribution by the author, apart from the coordination of the publication process, is minor compared to the contribution to the publications in Chapter A.

[6] Some points of entry into this topic can be found in Konstantinou (2006), in earlier publications by the author (Ranke and Jastorff, 2000; Ranke, 2001) and in Section A.10.

[7] Numerous reviews have been published on ionic liquids (see for example Welton, 1999; Wasserscheid and Keim, 2000; Plechkova and Seddon, 2008) that can be used as an introduction to the topic.

2 Concepts

2.1 Linear free energy relationships

For taking into account the behaviour of chemical substances after they have left the technically controlled systems of their use phase, it is important to be able to estimate the location of their equilibrium partitioning between the various phases in their "new" environment. Among such phases are air, inorganic surfaces, organic surface films, water, aerosol particles, and biological organisms, which may be subdivided into various tissues.

A common approach to estimating partition coefficients that carry the required information is to use Linear Free Energy Relationships (LFERs, see Schwarzenbach et al., 2003, p. 89ff). The use of such relationships for correlating the partitioning of molecular substances between water and 1-octanol, quantified by the partition coefficient $\log K_{\mathrm{ow}}$, and various other partition coefficients for two phase systems involving water is well established. Since the use and establishment of LFERs is a core part of this thesis, some theoretical background of their application especially to ionic liquids is given below.

In the following, $\log x_{i,\mathrm{w}}^{\mathrm{sat}}$ denotes the decadic logarithm of the mole fraction in the water rich phase of a hydrophobic substance i that forms a biphasic system with water.

If i does not only form a biphasic system with water, but is furthermore a neutral organic liquid, its activity coefficient with the pure substance as standard state $\gamma_i^{\bullet}$ can be approximated by $\left(x_{i,\mathrm{w}}^{\mathrm{sat}}\right)^{-1}$, provided that it only takes up a negligible amount of water, and this water does not significantly change the chemical environment within the pure substance (Schwarzenbach et al., 2003, p. 133ff).

If the activity coefficients $\gamma_i^{\bullet,j}$ – again with the pure substance i as standard state – in two non-aqueous phases $j \in \{1, 2\}$ are similar or even close to unity for a group of compounds, then an LFER can be established between the molar free energies of transfer $\Delta_{\mathrm{w}\to j}\ g_i$ between water and each of the two phases, in the form of

$$\log K_{i,1\mathrm{w}} = a_1 \log K_{i,2\mathrm{w}} + c_1 \tag{2.1}$$

where $\log K_{i,j\mathrm{w}}$ are the partition coefficients between water and phase j for substance i. Under the condition stated above, coefficient a will be around unity, and constant c gives information about the differences between the two phases j concerning molecular interactions with the set of substances chosen for the establishment of the LFER. a and c are determined by linear regression.

Such LFERs are used for estimating 1-octanol/water partition coefficients $\log K_{\mathrm{ow}}$ from thermodynamic capacity factors k' observed in isocratic reversed phase HPLC, *i.e.* using a fixed fraction ϕ of organic modifier in the eluent (OECD, 1989, and references cited therein):

$$\log K_{i,\mathrm{ow}} = a_2 \log k'_i + c_2 \tag{2.2}$$

In this equation, c_2 additionally includes a contribution of the phase ratio between mobile and stationary phase[1], and it strongly depends on the fraction ϕ of organic modifier used for establishing the correlation, while a_2 will again generally approximate unity.

In Figure 2.1, an overview of the measures used in this thesis for experimentally quantifying partitioning of the molecular organic isothiazol-3-one biocides, of ionic liquids, and of ionic liquid cations is given. LFERs are indicated by connecting lines.

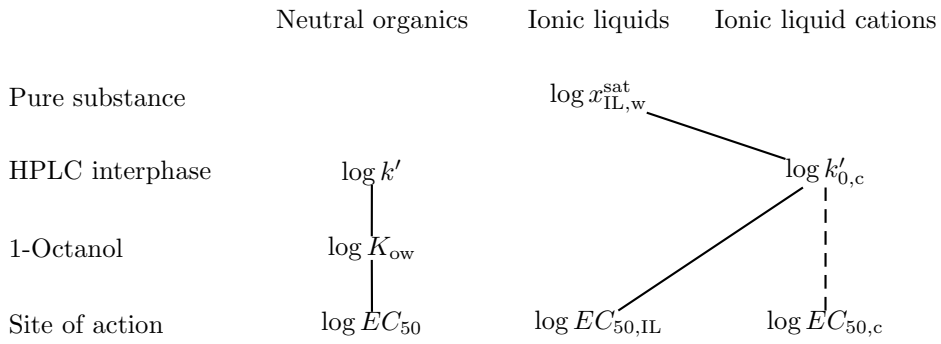


Figure 2.1: Measures for quantifying partitioning of chemical substances between aqueous phases and the phases listed on the left. Solid lines indicate LFERs between those measures, presupposing that the tendency to evade aqueous phases dominates partitioning. The rationale to view $\log \mathrm{EC}_{50}$ values as measures for partitioning between external aqueous phases and the assumed site of action is detailed in Section 2.2. The $\log \mathrm{EC}_{50}$ for cations is not observable directly, therefore the corresponding line for the LFER is dashed.

The correlation between $\log K_{\mathrm{ow}}$ and $\log k'$ given in Equation 2.2 was used for estimating 1-octanol/water partition coefficients for the isothiazol-3-one biocides (Section A.13).

Rationale and preconditions for correlating effect concentrations like EC_{50} values[2] (bottom row in Figure 2.1) with partitioning coefficients involving aqueous phases are given in Section 2.2.

In the case of ionic liquids, the situation is different in a number of ways. First of all, the molar free energy of transfer of the ionic liquid to water can be divided into separate

[1] The exact phase ratio in standard reversed phase HPLC can not be quantified, since the volume of the stationary phase, being an interphase between the solid silica gel and the liquid mobile phase, is ill-defined.

[2] The EC_{50} is the concentration at which a certain detrimental effect on an organism occurs at 50% of its theoretical maximum

contributions of its cation and its anion[3]. Secondly, because of the permanent charges on the ions, electroneutrality and electrostatic contributions to partitioning have to be taken into account.

Because of the importance of water in technical, biological and environmental systems, special attention has been paid by the author to determination and analysis of water solubility, as well as the partitioning of ionic liquid ions in biphasic systems where one of the phases is aqueous. A two-parameter LFER relating the water solubility of ionic liquids to the capacity factor of their cations as derived from gradient HPLC and a hydrophobicity descriptor for their anions is given in a recently submitted manuscript (Section A.14). The theoretical background for this two-parameter LFER is supplied in the Supporting Information to that manuscript.

2.2 Baseline toxicity and excess toxicity

Since the pioneering publications of Meyer (1899) and Overton (1901), the correlation between hydrophobicity or lipophilicity[4] and toxicity of nonreactive chemical substances has been described and reviewed many times (Franks and Lieb, 1990, 1994; Antkowiak, 2001). While the term narcosis has been established in the field of anaesthesia, describing unspecific reversible effects of mainly apolar compounds on mammals, in aquatic toxicology the term baseline toxicity has been coined for the correlation between lethal effect concentrations and lipophilicity(Könemann, 1981a). The common pattern of relating toxicity to lipophilicity suggests that baseline toxicity and narcosis are closely related, which is generally accepted in aquatic toxicology.

A distinction between the baseline toxicity of apolar narcotics (narcosis type I, general or apolar narcosis) and polar narcotics (narcosis type II, polar narcosis) has been proposed (Saarikoski and Viluksela, 1982; Veith and Broderius, 1990), and there is an ongoing debate about the underlying mechanisms and whether there really is a difference (Franks and Lieb, 1994; Vaes et al., 1998; Roberts and Costello, 2003b). In this thesis, the distinction is not used.

The quantification of lipophilicity or hydrophobicity in such studies is most often based on the partitioning coefficient $\log K_{\mathrm{ow}}$ between water and 1-octanol, which can be directly measured, indirectly determined by reversed phase HPLC, or estimated by various computational methods.

Once a baseline toxicity correlation has been established, it can be used to discriminate excess toxicity of substances that exhibit higher toxicity than predicted by the baseline correlation. A distance of one log unit from the regression line, equivalent to a Toxic Ratio (TR) of 10 has been proposed for distinguishing substances with excess toxicity from narcotics/baseline toxicants (Verhaar et al., 1992, 2000; Maeder et al., 2004).

An interesting observation for a mechanistic interpretation of baseline toxicity has

[3] Generally, an ionic liquid is composed of a monovalent cation and a monovalent anion

[4] In earlier contributions, the author has preferred the term lipophilicity (Section A.5). Meanwhile, the author prefers the term hydrophobicity, in cases where the second phase seems to be of minor importance for the location of the partitioning equilibrium.

been made by McCarty et al. (1991, 1993): Because bioconcentration of neutral organic chemicals in aquatic organisms quantified as $\log \mathrm{BCF}$ positively correlates with $\log K_{\mathrm{ow}}$ in a certain interval with a slope around unity, and toxicity, quantified as $\log \mathrm{EC}_{50}$ correlates with a slope around negative unity, it can be postulated that the accumulated concentration at an effect level of 50% is approximately constant. As a matter of fact, evaluation of a large body of literature showed that for baseline toxicants with an unspecific mode of action, a critical body residue (CBR) between 2 and 8 mmol/kg wet weight is typical for acute aquatic toxicity in small fish (McCarty et al., 1992). The reasons for excess toxicity are commonly assumed either to result from reactivity or from sterically specific interactions with specific molecular targets (Escher and Hermens, 2002).

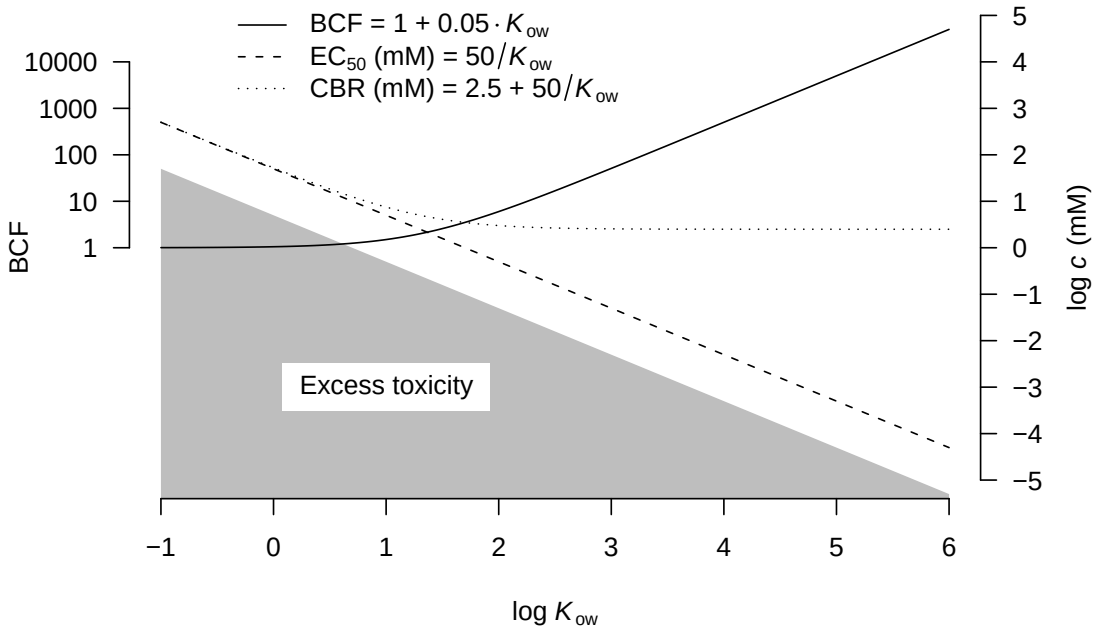


Figure 2.2: Idealized scheme of the relation of bioconcentration (BCF), toxicity (EC_{50}), and critical body residue (CBR) for small fish using the equations proposed by McCarty et al. (1992). The grey area covers EC_{50} values that are more than 10 times smaller than expected from 1-octanol/water partitioning.

The idealized relations shown in Figure 2.2 suggest, that the CBR should actually decrease for baseline toxicants with very low hydrophobicities. However, this has so far not been empirically shown to the knowledge of the author.

The assumption of a constant concentration of baseline toxicants that causes a certain effect level at the site of action (a constant internal effect concentration) qualifies the

baseline toxicity relation

$$\log \mathrm{EC}_{50} = -a_3 \log K_{\mathrm{ow}} + c_3 \tag{2.3}$$

as an LFER, because the EC_{50} can be regarded as the concentration of the substance in the aqueous phase surrounding the organism which is at equilibrium with this internal effect concentration. This thermodynamic interpretation of baseline toxicity is in line with the proposal of Ferguson (1939) to use the chemical potential as an indicator of toxicity. Values of the slope parameter a_3 around unity can be taken as an indication for equilibrium governed toxicity[5].

The baseline toxicity relation can now be transformed to use the thermodynamic capacity factor k' for quantifying hydrophobicity, by inserting Equation 2.2 into Equation 2.3. This means, that the partitioning between mobile phase and stationary phase in HPLC is related to the partitioning between the surrounding aqueous medium of an organism and the site of action for an observed effect.

$$\log \mathrm{EC}_{50} = -a_4 \log k' + c_4 \tag{2.4}$$

The meaning of excess toxicity for a set of selected reactive isothiazol-3-one biocides covering a wide range of hydrophobicities was experimentally investigated and discussed in Section A.13.

A new type of baseline toxicity relationships for organic cations such as used in ionic liquids was established by the author at the example of a mammalian cell line (Section A.5 and Section A.6), several aquatic organisms (Section A.8) and even for inhibition of acetylcholinesterase (Section A.9) using a gradient reversed phase HPLC derived hydrophobicity parameter that is described in Section 3.1.

An overview of these relationships and their interpretation can be found in Section 4.2.

2.3 Basal cytotoxicity

The term basal cytotoxicity was coined by Ekwall (1983, 1995), addressing toxicity of chemicals to basal functions of all cells of an organism, as opposed to organ specific toxicity and extracellular toxicity.

Basal cytotoxicity initially assessed in mammalian cells has been reported to be similar in and therefore relevant to such different organisms as plants (Kristen, 1997) and fish (Castano and Gómez-Lechón, 2005) and under certain conditions, correlations to in vivo data have been found to be of predictive value (Garle et al., 1994).

The author proposes that cytotoxicity tests in largely dedifferentiated cancer cell lines such as the IPC-81, cell line excessively used in this thesis (Section A.1 and the following publications) provide a convenient screening method for obtaining first rough estimates for the toxic potential of relatively large sets of substances. Metabolically active cell lines like Hep G2 are often preferred in toxicity screening. However, the increased presence

[5] In practice, values for a_3 are oftentimes smaller than 1 (see Hermens et al., 1985, for example)

of metabolic enzymes in these liver cells isolated from liver is already a departure from the minimum or basal features of a cell. Of course, no real cell type will ever qualify for assessing basal toxicity on its own, but rather, combinations of different cells should be used, as demonstrated in the publications by Ekwall and — to some degree — in this thesis.

Comparisons of baseline toxicity equations and excess toxicities of ionic liquids and antifouling biocides in mammalian cell lines and aquatic organisms are summarized in Sections 4.2 and 4.1.

The concept of basal cytotoxicity is not principally related to the concept of baseline toxicity. However, it can be assumed that non-reactive, unspecifically acting substances will exhibit baseline toxicity as well as basal cytotoxicity. The probability of observing basal cytotoxicity and baseline toxicity depending on chemical interaction types is shown in Figure 2.3.

	reactive interaction(s)	stereo-specific interaction(s)
baseline toxicity	unlikely[a]	unlikely
basal cytotoxicity	likely	tissue/taxonomy dependent

Figure 2.3: Probability of the observation of basal cytotoxicity and baseline toxicity depending on interaction types of a toxicant.[a]but see the example given in Section 4.1

2.4 Mixture toxicity

In pharmacology, toxicology, and recently also in the field of environmental risk assessment, theories describing effective dose levels of mixtures in terms of components have been developed. The two most prominent types of quantitative relations in this respect are

- Concentration Addition (CA) or "Loewe additivity" (similar mode of action) and
- Independent Action (IA) or "Bliss independence" (different mode of action)

as reviewed by Boedeker et al. (1993) and Greco et al. (1995). The quantitative relation between predictions base on these two theories depends on the number of components in the mixture, the concentration ratio of mixture components, the response level under consideration, and the slopes of the concentration-response curves of individual toxicants (Boedeker et al., 1993; Drescher and Boedeker, 1995). In many environmentally relevant cases, predictions made by CA are conservative in the sense that they produce higher mixture toxicity, but under some circumstances, the reverse can be the case, or the predictions can be indistinguishable (Drescher and Boedeker, 1995; Backhaus et al., 2004).

It has been shown, that the toxicity of mixtures of baseline toxicants is consistent with the CA model (Könemann, 1981b; Hermens et al., 1984, 1985).

Because of the successfull correlation of ionic liquid toxicity with cation hydrophobicity (Section A.5, Section A.6) it was attempted to extend the model by tentatively assuming a similar unspecific mode of action of cation and anion.

The CA model for a given effect level of 50% and a binary mixture can be denoted as

$$\frac{c_1}{\mathrm{EC}_{50,1}} + \frac{c_2}{\mathrm{EC}_{50,2}} = 1 \tag{2.5}$$

Because we can assume that in a solution of an ionic liquid the concentration of cation and anion will be equal, we use $c = c_1 = c_2$ and arrive at

$$c = \frac{\mathrm{EC}_{50,1} \cdot \mathrm{EC}_{50,2}}{\mathrm{EC}_{50,1} + \mathrm{EC}_{50,2}} \tag{2.6}$$

which is valid at this effect level. Because the molar concentration of the IL at this effect level is just equal to the concentration of anion and cation and therefore equal to c, we obtain as a testable hypothesis for the toxicity of the ionic liquid

$$\mathrm{EC}_{50,\mathrm{IL}} = \frac{\mathrm{EC}_{50,\mathrm{c}} \cdot \mathrm{EC}_{50,\mathrm{a}}}{\mathrm{EC}_{50,\mathrm{c}} + \mathrm{EC}_{50,\mathrm{a}}} \tag{2.7}$$

where the cation effect concentrations $\mathrm{EC}_{50,\mathrm{c}}$ can be quantified by a halide salts of the cations, and the anion effect concentrations $\mathrm{EC}_{50,\mathrm{a}}$ can be quantified by sodium or lithium salts of the anions, assuming that these counteranions do not significantly influence toxicity.

The first results of testing this hypothesis using the IPC-81 cell culture are reported in Section A.3. An update of these findings is given in Section 4.2.

2.5 Indicators for more sustainable chemistry

2.5.1 Ecotoxicological risk indicators for chemical substances

Comparative ecotoxicological risk profiles of chemical substances that can alternatively be used in a certain application have been previously proposed and applied to antifouling biocides (Ranke and Jastorff, 2000; Ranke, 2001; Ranke and Jastorff, 2002). They are inspired by the work of Scheringer and Müller-Herold with their respective co-workers on persistence and spatial range for the evaluation of chemical substances in accordance with the precautionary principle, as well as by the idea of an assessment of the hazard potential of a chemical by persistence, bioaccumulation and toxicity (PBT assessment). However, a risk presupposes the probability of a release of a substance to some uncontrolled environment, and this release probability can already be taken as a first risk indicator when comparing substances. Furthermore, in many situations it is desirable to compare substances with highly varying data availability or specific uncertainties. Therefore, the five risk indicators release, spatiotemporal range, bioaccumulation, biological activity, and uncertainty were defined as essential components of an ecotoxicological risk profile (Ranke, 2001).

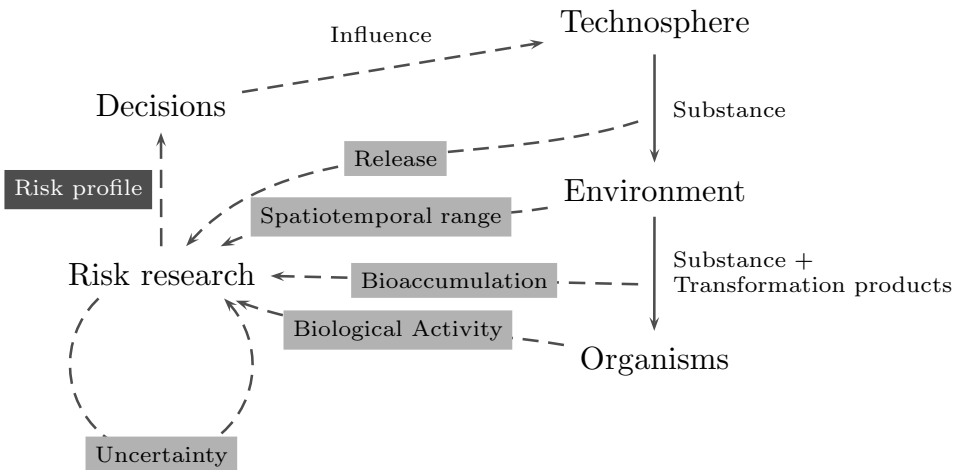


Figure 2.4: Risk assessment cycle involving comparative ecotoxicological risk profiles consisting of two (Release and Uncertainty) up to five risk indicators. Note the growing complexity of the risk assessment when proceeding from a release based assessment to a complete risk profile including fate, bioaccumulation and effects of transformation products.

Figure 2.4 shows how ecotoxicological risk profiles can form an essential element in the risk management cycle for chemical products.

In the course of a description of the product design strategy of the complete ionic liquid team of the Centre for Environmental Research and Sustainable Technologies (UFT) of the University of Bremen, the concept was applied by the author to a preliminary comparison of two room temperature ionic liquids with the conventional solvent acetone (Jastorff et al., 2003a). These preliminary risk profiles where later updated (Ranke et al., 2005).

The same risk indicators were used as a structural basis for a review on ionic liquids as sustainable products (Section A.7). In this review, the ideas of joint bioaccumulation and joint biological activity, that were first presented in a conference on internal exposure in 2004 (see the poster reproduced in Section B.5) are integrated into the indicator definitions, and thus it may serve as the current definitive reference.

2.5.2 Indicators for chemical reactions

In the course of a project striving at improving the sustainability in the organic chemistry lab courses taught at German universities (Nachhaltigkeit im organisch-chemischen Praktikum, NOP), indicators for the efficiency of chemical reactions were sought. The following is an edited version of the section "Simple indices" of the NOP article Indices for chemical reactions written by the author.

In synthetic organic chemistry yield and purity are common indices, characterizing the

quality of a chemical transformation. The yield Y is defined as the quotiont of the actual amount of product obtained from the reaction (actual yield) and the amount of product which could have theoretically been obtained if the total of limiting reagent R had been transformed according to the stoichiometric equation (theoretical or stoichiometric yield), presuming 100 % purity of the reagents.

If n_K is the amount of the limiting reagent before the reaction and a_P and a_K are the stoichiometric coefficients of product P and limiting reagent R, the yield is given by

$$Y = \frac{n_\mathrm{P} a_\mathrm{R}}{n_\mathrm{R} a_\mathrm{P}}. \tag{2.8}$$

If n_P is the amount after purification, it is called the final yield. Generally, the yield defined by this equation will be expressed as a percentage (percent yield or percentage yield).

Before actually performing the reaction, one can already derive the so-called atom economy from the stoichiometric equation. The atom economy expresses which fraction of the summed atomic masses on the left hand side of the equation appear in the product. By virtue of this definition, it is an evaluation of a synthetic approach from an economic point of view. The concept of atom economy was introduced by Trost (1991, 1995b,a).

The mass efficiency of a reaction we define as the ratio of the mass of the obtained and purified product m_P divided by the sum of the masses of all substances introduced to a reaction mixture during the experiment:

$$e_\mathrm{S} = \frac{m_\mathrm{P}}{\sum_i m_i} \tag{2.9}$$

where index i runs over all used substances. Coolants like cooling water or ice, not commixing with the reaction mixture are not taken into consideration accoring to our convention. Accounting for such substances is the task of the more detailed method of input analysis.

Mass efficiency e_S as defined here is similar to the inverse of the E factor (environmental factor) introduced by Sheldon (1994), or exactly the inverse of the mass index S^{-1} as defined by Eissen and Metzger (2002). The advantage of the efficiency measure is that a higher indicator value actually indicates an improvement. On the other hand, a graphical representation of the different input materials m_i is often desirable. The use of the mass index for this purpose is described in Section 3.3.1.

The energy efficiency e_E of a reaction is defined in analogy as the ratio of the mass of the obtained and purified product m_P divided by the sum of the energy consumed during the experiment:

$$e_\mathrm{E} = \frac{m_\mathrm{P}}{\sum_k E_k} \tag{2.10}$$

where index k runs over all separately measurable energy consumptions, i.e. in general the consumed electrical energy. In this measure the energy used to produce ice for cooling is included. Important hints for the measurement of such energy contributions are presented in the NOP article Energy metrics and measurements.

Atom economy, mass efficiency and energy efficiency are automatically calculated for the NOP experiments and can be found under menu entry "Evaluation", submenu entry "Indices" on the page of each experiment[6].

[6]Example link: www.oc-praktikum.de/en-experiment-2003-evaluation-indices

3 Tools

In the following, the most important tools that have been developed in the area covered by this thesis are described. Section 3.1 describes the hydrophobicity determination method that was proposed in the paper reproduced in Section A.5. In Section 3.2 an overview of the information technology that was developed in order to make it possible to store and evaluate a large amount of bioassay data in a standardized way is given.

Section 3.3 is description of the interactive databases that were created by the author together with Dr. R. Störmann, in the context of the new and more sustainable organic chemistry lab course (Subsection 3.3.1) and the ionic liquid development project (Subsection 3.3.2), focussing on the parts created by the author. The contents of Sections 3.2 and 3.3 have not been published before.

3.1 Hydrophobicity determination of ionic liquid ions

As described in Section 2.2, the quantification of lipophilicity in narcosis related studies is most often based on the partitioning coefficient $\log K_{\mathrm{ow}}$ between water and 1-octanol, which can be directly measured, indirectly determined by reversed phase HPLC, or estimated by various computational methods.

In surfactant science (Roberts and Costello, 2003a), and recently also in the ionic liquids literature (Stepnowski and Storoniak, 2005), $\log K_{\mathrm{ow}}$ values are in many cases predicted from their structure, based on the group contribution method of Hansch and Leo (Hansch et al., 1995). This works well within groups of ionic liquids sharing the same basic ionic structure and the same counter ion (e.g. benzalkonium chlorides, 1-alkyl-3-methylimidazolium tetrafluoroborates). However, an absolute prediction for different ionic headgroups is not possible, since the necessary fragment constants for the headgroups are not available.

The absence of such fragment constants is related to problems encountered when determining 1-octanol water partitioning constants for ionic substances. On the one hand, ions with surfactant properties will disturb phase separation in the 1-octanol water system. On the other hand, the counter anion might have an influence on partitioning. The second problem can be avoided by keeping the anion constant, and ensuring sufficient deionization of the water employed, but the problem of phase separation which is already an issue for neutral substances because of the formation of microdroplets in the aqueous phase (Tolls et al., 2003), is not easily overcome.

Different methods have been proposed to calculate lipophilicity parameters from a single gradient HPLC run (Kaune et al., 1995; Snyder and Dolan, 1996; Krass et al., 1997; Valko et al., 1997). The approaches of Kaune et al. (1995) and Valko et al. (1997) are

designed to predict $\log K_{ow}$ values, but depend of the availability of a structurally related reference set of reliable $\log K_{ow}$ data that have been directly determined. Furthermore, it has been shown that under some circumstances, chromatographic parameters are even better descriptors of bioavailability, including toxicity, than octanol/water partitioning coefficients (Hsieh and Dorsey, 1995).

Therefore, a quick chromatographic method for characterizing the lipophilicity of cations and maybe for anions was sought. While there have been extensive efforts to characterize ionic liquids as stationary phases by chromatography as reviewed by Poole (2004), the use of gradient HPLC for the characterization of ionic liquids as solutes is to our knowledge unprecedented. The use of isocratic HPLC is not feasible, because of the large range in hydrophobicities covered by the ionic liquid cations to be investigated.

A point was made to derive a lipophilicity measure that is linearly correlated with thermodynamic hydrophobicity. It is clear from available theoretical treatments of gradient HPLC that neither the retention time determined in gradient HPLC (Valko et al., 1997), the so-called capacity factor derived from gradient HPLC as if it were isocratic HPLC used by several authors (Kaune et al., 1995; Krass et al., 1997; Paschke et al., 2001), nor any parameters derived by linear transformations like the chromatographic hydrophobicity index (CHI) proposed by Valko et al. (1997); Valko (2004) fulfill this requirement. The nonlinear relation of gradient "capacity factors" with $\log K_{ow}$ has been reported by Kaune et al. (1995); Paschke et al. (2001). On the other hand, the gradient retention times determined in fast gradients were reported to yield linear correlations with $\log K_{ow}$ values to a satisfactory degree.

However, the perspective to obtain a parameter that is in theory linearly related to thermodynamic hydrophobicity lead the author to the procedure described below, because of the possibility to use it as a basis for the LFERs described above (Section 2.1).

The retention behavior in reversed phase chromatography is often described by the linear solvent strength (LSS) model

$$\log k' = \log k'_w - S\,\phi, \tag{3.1}$$

describing the dependence of the capacity factor $k' = \frac{t_R - t_0}{t_0}$ on the fraction of organic solvent (ϕ) in the mobile phase, with k'_w ideally describing the capacity factor at zero percent organic solvent, i.e. an aqueous phase. t_R is the retention time and t_0 is the dead time of the system. The slope factor S shows the influence of the organic modifier on k' and is generally also substance dependent. k', S and ϕ are dimensionless numbers and ϕ ranges from 0 to 1 (inclusive).

k'_w can be derived from many observations of $\log k'$ at different values of ϕ by linear regression according to Equation 3.1. The good correlation of $\log k'_w$ values for reversed phase HPLC with $\log K_{ow}$ within a group of sufficiently similar neutral organic substances is long-established, *e.g.* by Braumann and Jastorff (1986); Ritter et al. (1995). For eight ionic liquid cations, $\log k'_w$ values have been successfully correlated to theoretically predicted $\log K_{ow}$ values by Stepnowski and Storoniak (2005).

For an estimation of k'_w for a set of substances with widely varying retention behavior,

two or more gradient runs can be used (Snyder and Dolan, 1996). If two different gradient runs are used, the two unknown k'_w and S values can be calculated from the data using the equations

$$t_R = \frac{t_0}{b} \log(2.3\, k'_0\, b\, (1-x) + 1) + t_0 + t_D \tag{3.2}$$

and

$$b = \frac{V_m\, \Delta\phi\, S}{t_G\, F} \tag{3.3}$$

where t_0 is the column dead time, t_D is the equipment dwell time, b is the dimensionless gradient slope, k'_0 is the capacity factor at the beginning of the gradient, V_m is the column dead volume, t_G is the gradient time, F is the flow velocity, $\Delta\phi$ is the change in the fraction of organic solvent during the gradient, and $x = t_D/(t_0\, k'_0)$ is the fractional migration through the column during pre-elution. In the following, the gradient always starts with 100 % aqueous phase ($\phi_0 = 0$) *i.e.* k'_0 is equal to k'_w. Note that Equation 3.2 is only valid if $t_R < t_G + t_0 + t_D$ (Snyder and Dolan, 1998).

According to a method initially established by de Galan and co-workers (Schoenmakers et al., 1981) and later refined by Snyder and Dolan (1996), k'_0 can also be estimated from a single gradient run if a correlation between k'_w and S is established according to the equation

$$S = p + q\, \log k'_w \tag{3.4}$$

for a subset of the substances in question with S and k'_w derived either from Equation 3.1 or from two or more gradient runs and Equation 3.2. In order to develop a method that covers a wide range of hydrophobicities, several gradient runs are used to derive S and k'_w for a series of reference compounds.

Coefficients p and q are obtained as intercept and slope of a linear regression of S against $\log k'_w$ for different substances. This then leads to the possibility to calculate k'_0 directly from t_R obtained in one gradient run by inserting Equation 3.4 into Equation 3.2. Experimental details of the method can be found in Section A.5.

In this way, $\log k'_0$ for 55 inert cations were determined. Additionally, in a period where the gradient equipment of the HPLC system was defective, $\log k'_0$ values were determined for eight further cations by linear calibration of isocratically measured k'_0 values with the ones from the original method. Therefore, a consistent dataset on 63 cations, out of which 60 have been used as componens of ionic liquids, is now available.

3.2 Automation in toxicity data handling

In the course of the data generation for the first publication of the author and co-workers reproduced in Section Section A.1 it became clear that standardized procedures not only for data generation in the form of bioassay protocols were necessary, but also for data

storage and processing. This was especially the case because of the many researchers that were involved in these processes.

It was furthermore desirable to create a central storage for the raw data for the team, with the possibility of distributed entry and review of data from various personal computers, so the team members could join their efforts by working on a common database, and plan their experiments accordingly.

Because of the previous positive experience of the author with interfaces of relational MySQL databases written in the scripting language PHP, these tools were chosen for this task.

3.2.1 Forms for entering data

Some of the bioassays used in the first characterizations of ionic liquids were based on multiwell plate readers. For these assays, input forms were generated in the PHP scripting language that would allow for convenient specification of the experimental design chosen. An example screenshot showing test design specification and data upload with a web browser[1] for a WST-1 96 well plate assay carried out with the IPC-81 cell line is shown in Figure 3.1.

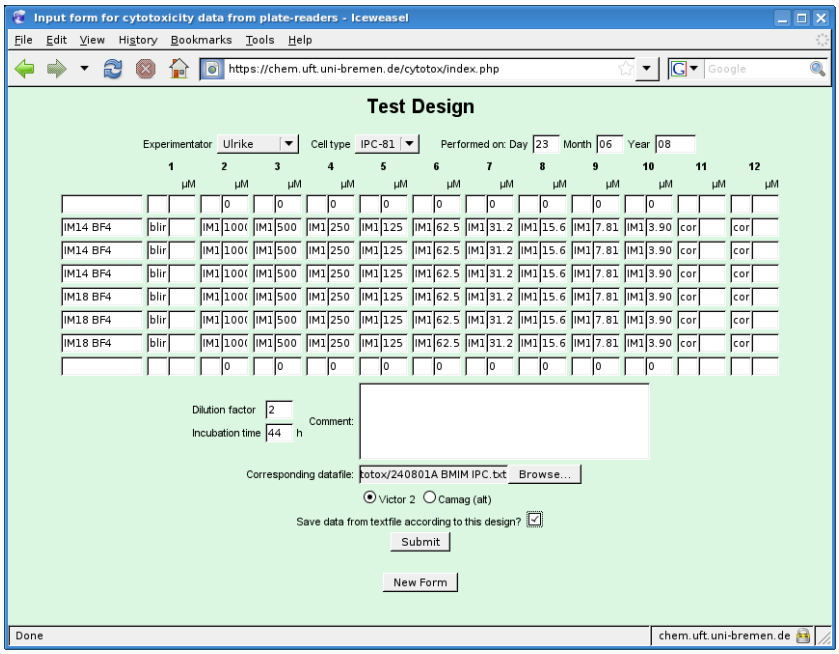


Figure 3.1: Data upload using a web browser with a test design specifying three parallel dilution series for two different ionic liquids.

[1] The iceweasel browser employed in the screenshot is just the free derivative of the standard firefox browser created by the Debian project. Firefox, Internet Explorer and Konqueror web browsers can be used as well.

During the upload of the text file, the raw data from the text file is associated with the data from the test design specified in the form and the response for each data point is calculated from the absorbance of the corresponding well according to the formula

$$r = \frac{A_i - \bar{A}_{\text{blind}}}{\bar{A}_{\text{control}} - \bar{A}_{\text{blind}}} \tag{3.5}$$

where r denotes the response of the bioassay, normalized to the interval from 0 (no response) to 1 (response of untreated controls) as defined in Section A.4, A_i is the absorbance in the well in presence of the tested substance at the specified concentration, $\bar{A}_{\text{blind}}$ is the mean absorbance of the wells without cells, and $\bar{A}_{\text{control}}$ is the mean absorbance of untreated control wells.

The raw data of blind and control wells, as well as the calculated response values are immediately written to the MySQL database holding the bioassay raw data, and a report is being generated, listing all the data entered, so the uploader can control the correctness of the operation.

A similar form was created for entering data from enzyme inhibition assays, with the difference that the response r of the enzymatic transformation was calculated using kinetic constants for the change of absorbance over time as provided by the plate reader.

At first, data generated in the widespread luminescence inhibition assay with *Vibrio fischeri*, were collected using spreadsheet software. In order to generate a comprehensive and consistent pool of bioassay data, this data was later transferred to an analogous MySQL database for raw data.

For entering new data into this MySQL database, another type of data entry form was created, where the numerical raw data from the experiments is not taken from a text file generated by a multiwell plate reader, but is entered directly by the user. The data entry form generated for the *Vibrio fischeri* is shown in Figure 3.2.

The data entered in the *Vibrio fischeri* form were evaluated as follows, before writing to the database: At first, the mean $\bar{R}_{\text{control}}$ of the ratio of the light intensities in the beginning (I_0) and at the end (I_1) of the exposure period

$$\bar{R}_{\text{controls}} = \frac{1}{n} \sum_n \frac{I_1}{I_0} \tag{3.6}$$

is calculated. The response r written to the database is then calculated as the ratio of the light intensity ratio under the specific exposure conditions divided by the light intensity ratio of the controls $\bar{R}_{\text{controls}}$.

Data evaluation for the *Lemna minor* growth inhibition test with an exposure period Δt of seven days was based on growth rates either calculated from the increase in frond number μ_{fronds} or on frond area μ_{area} (Figure 3.3)

$$\mu_{\text{fronds}} = \frac{\ln n_i - \ln n_0}{\Delta t} \tag{3.7}$$

$$\mu_{\text{area}} = \frac{\ln A_i - \ln A_0}{\Delta t} \tag{3.8}$$

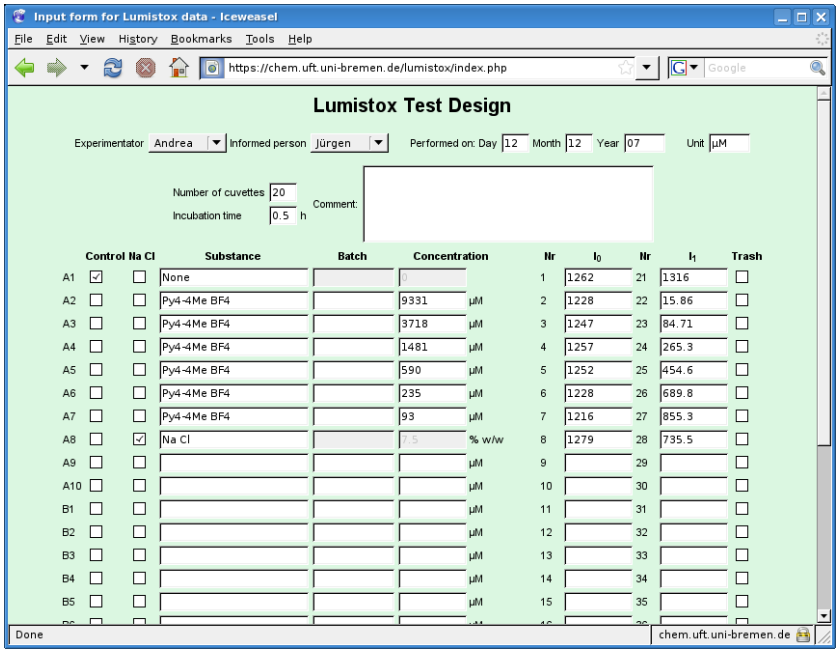


Figure 3.2: Data entry form for the bioassay with *Vibrio fischeri*. Note that the form is not completely filled. The entered data just serves for illustration purposes.

where n_0 and n_i are the frond numbers at the beginning and at the end of the exposure period, and A_0 and A_i are the frond areas at the beginning and at the end of the exposure period, respectively. The response r is then calculated as the growth rate of the treatment divided by the growth rate of the control.

For the *Scenedesmus vacuolatus* bioassay, the response written to the database was calculated as the reproduction ratio of the treatments divided by the reproduction ratio of the controls, where the reproduction ratio R is calculated from the algal cell densities according to

$$R_i = \frac{\bar{d}_i - \bar{d}_0}{\bar{d}_0} \tag{3.9}$$

where $\bar{d}_i$ and $\bar{d}_0$ are the mean cell densities at the end of the 24 hour exposure period of the treatment and all controls, respectively. The four replicates for each data point (compare Figure 3.4) consist of two replicate counts of two replicate assay tubes.

3.2.2 Quality control and dose-response curve evaluation

Because of its quality, wealth of core and contributed functions, and availability for all major computing platforms, the R system is becoming more and more a *de facto* standard for statistical computing in academia, but is also used in large private companies like Pfizer (Kuhn, 2006).

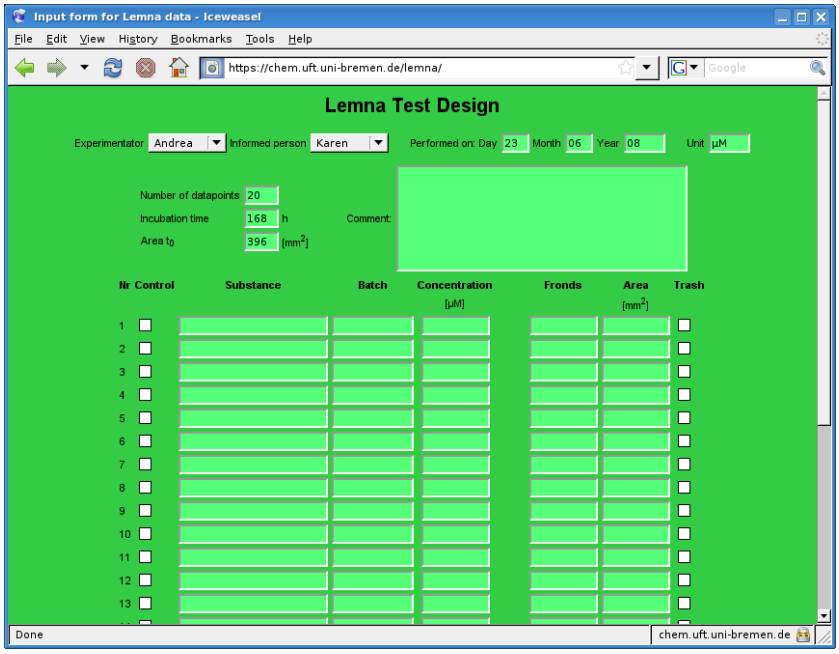


Figure 3.3: Data entry form for the bioassay with *Lemna minor*.

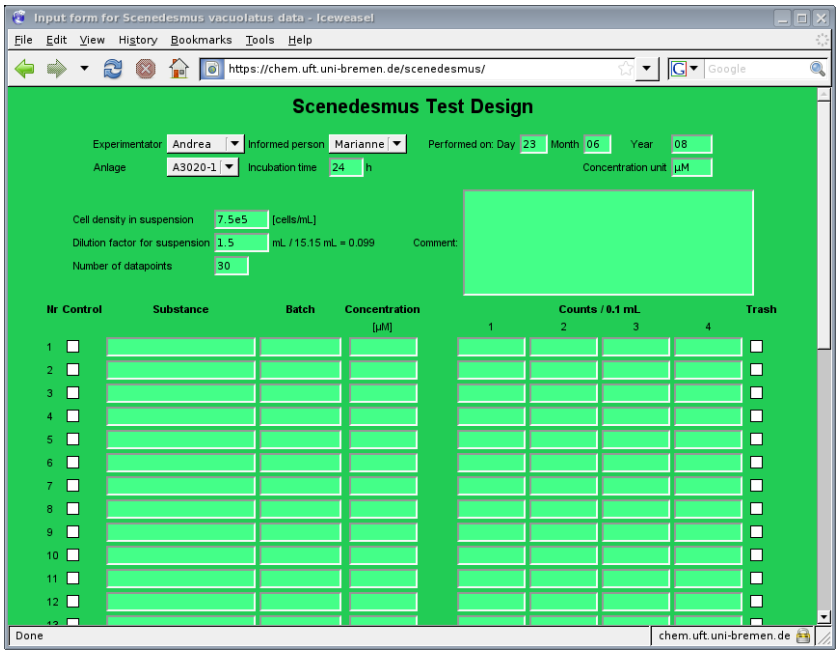


Figure 3.4: Data entry form for the *Scenedesmus vacuolatus* bioassay.

Here, the `RODBC` extension to R is used for retrieving data from local and remote MySQL databases, for data analysis as well as for graphical presentations of the results. The functions described in the remainder of this section have been published in the Comprehensive R Archive Network (CRAN) (Ranke, 2007a) and are actively maintained since July 2004.

Once the raw data and the calculated response values are in the database, the data from each experiment has to be carefully reviewed before it is considered valid. For this purpose, the `checkplate` and `checkexperiment` functions were created. They retrieve the raw data from a specified experiment from the respective MySQL database, and present the data, as well as some statistics on the controls (Figure 3.5).

If the absolute values and relative standard deviations of the controls in the experiment satisfy the given conditions, and a clear concentration dependence of the response is visible, the data are marked valid in the database.

The `drfit` function for dose-response curve fitting, which is capable of doing probit, logit, weibull and linlogit fits to the data, as well as the `drplot` function providing a flexible way to plot the results from the fitting procedure are described in Section A.4.

3.3 Interactive databases for improving sustainability

The integration of environmental factors into the development of chemical products and processes as early as possible (see for example Heinzle and Hungerbühler, 1997; Weidenhaupt and Hungerbühler, 1997; Koller et al., 2000; Hungerbühler et al., 1998) depends on the availability of relevant information on probable or certain environmental impacts.

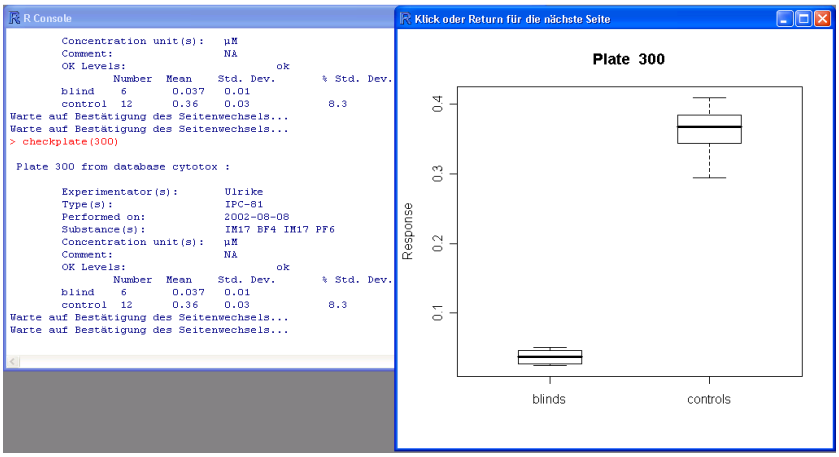
The motivation for creating the tools described in the following two subsections was to increase the quality of risk relevant information in terms of available quantity, immediacy of retrieval, homogeneity, clarity, and source attribution.

The two database driven web applications described are in a different stage of development, and serve different purposes. The website to the NOP project, providing more than 80 organic synthesis experiments together with background data on sustainability, including risk relevant data on the substances employed, was first published in German in 2003. An almost completely rewritten version featuring English and a partial Italian translations was put online in 2005, and is still complemented with new features. The substance data are kept up to date, and translations to further languages are currently being completed as described in Section A.12.

The UFT internal interactive database on ionic liquids (UFT-ILDB) has been and is actively being used by the ionic liquids team of the UFT. It aims at internally providing consistent naming, housekeeping and quality assurance for ionic liquids and ionic liquid precursors studied by the team, while simultaneously collecting the properties of these substances generated by the team.

Both databases share some underlying technology, the substance database part even shares some PHP source code, and the MySQL database table definitions are similar. Another technique that they commonly use is the version control system. While the

a)



b)

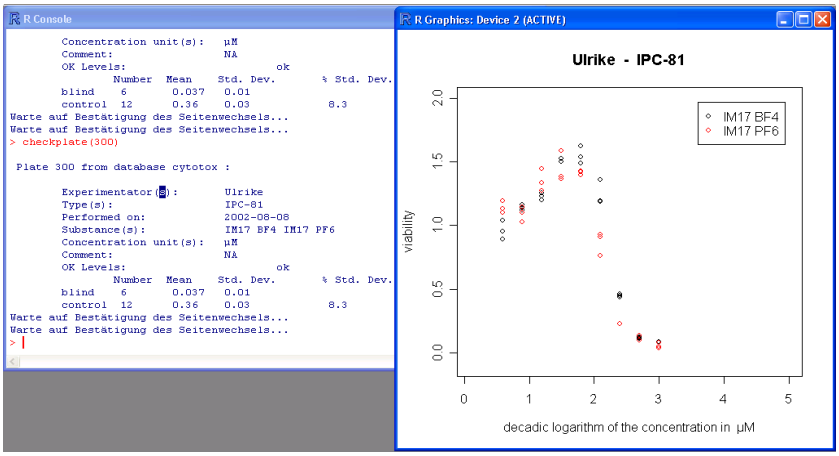


Figure 3.5: Data quality control at the example of two ionic liquids tested in the IPC-81 assay using the WST-1 reagent. In a first plot (a) box plots of the control data without cells (blinds) and without toxicant (controls) are shown. In a second plot (b), the response r of all data points is plotted against the logarithm of the concentration. To the left (both screenshots) a summary of the dose-response experiment is given.

source code for the UFT-ILDB has not been published, the source code for the NOP website has been made available via the version control repository located at the UFT. The present state of the website code and all revisions back to the beginning of the rewrite in January 2004 can be accessed via the web interface to the version control system at kriemhild.uft.uni-bremen.de/viewcvs/www.

3.3.1 Application in chemical education

Among the aspects of the database driven interactive website of the NOP project (www.oc-praktikum.de) that are described in Section A.12 are views of the required substances for a specific experiment, showing the required amount of each substance, its hazard symbols, and its R and S phrases according the European classification of dangerous substances[2], with a colored background according to data availability[3] and according to effect factors according to the German TRGS 440[4].

Without going into the details of the database of the chemical substances that can best be explored online, only the new feature of the graphical representation of the mass index is described here.

Figure 3.6 shows the two version of interactive bar charts showing the contributions of the different input materials to the synthesis of one gram product for the 0.1 mol version of NOP experiment 2003. Such bar charts are automatically generated for each of the experiments in the database.

While the generation of this and other types of bar charts for organic synthesis procedures has been possible since the publication of the EATOS software [5] by Marco Eissen and co-workers, the integration of such charts in the website has several advantages:

- It is not necessary to install additional software[6].
- Mass index graphs are immediately available for all of the experiments in the NOP database.
- The colored backgrounds provide an overview of knowledge on (data availablility) and hazard potential of (effect factors) the substances employed in an experiment, visually weighted by their quantities.
- The segments of the bar chart are hyperlinks to the substance page of each of the substances employed, so the data base for the different colors can be explored.

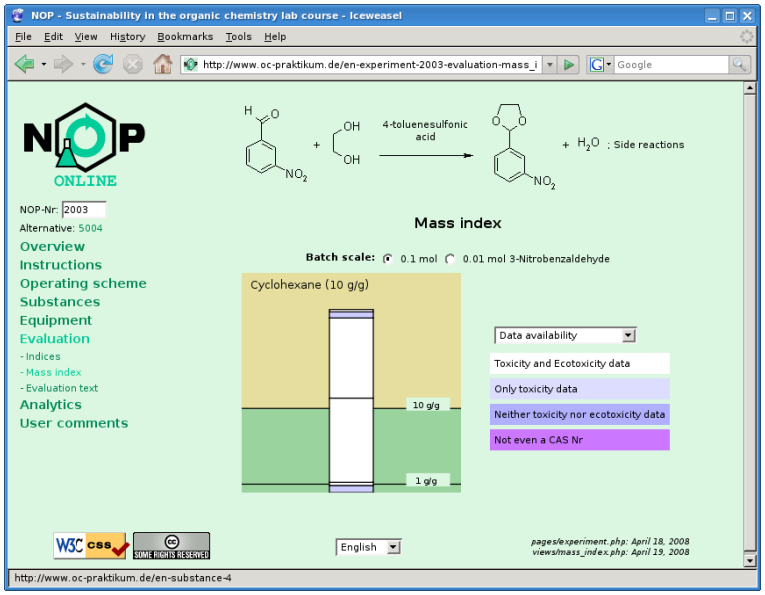
On the other hand, the scope of the mass index representations is limited to the NOP database, and depends on the quality of its data, so it is clear that both approaches are complementary and should be further developed.

3.3.2 Application in chemical product development

Certainly, the collection of information about chemical substances in a database is not a novel idea. However, the author is not aware of any academic groups working on the

[2]Example link: www.oc-praktikum.de/en-experiment-2003-substances-required
[3]Example link: www.oc-praktikum.de/en-experiment-2003-substances-data_availability
[4]Example link: www.oc-praktikum.de/en-experiment-2003-substances-effect_factors
[5]EATOS is freely available from www.chemie.uni-oldenburg.de/oc/metzger/eatos
[6]This is true when Mozilla Firefox, Opera, Safari, or Konqueror web browsers are in use, because they provide native support for Scalable Vector Graphics (SVG) that are used in this solution. Users of Internet Explorer have to install the Adobe SVG viewer plugin.

a)



b)

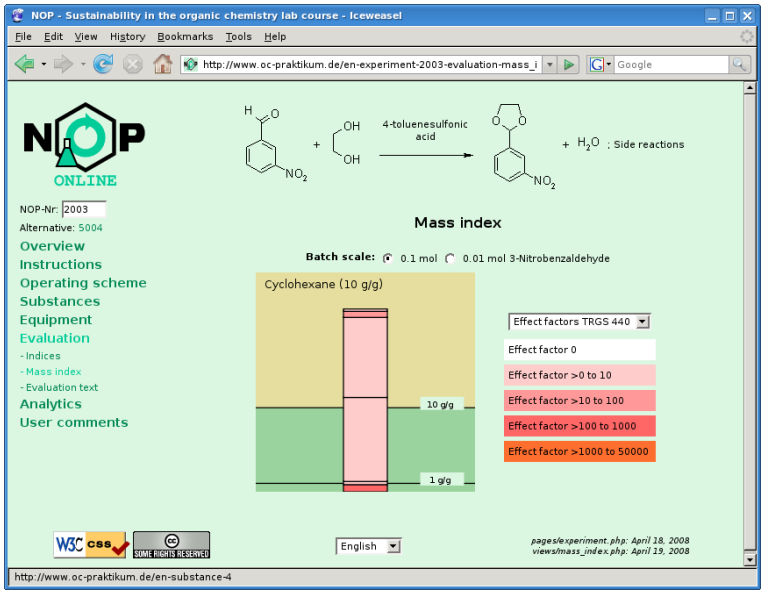


Figure 3.6: Mass index graphs illustrating the contributions to the mass index colored according to a) data availability and b) effect factors of the input materials. Note that chemical name and mass index contribution for each substance are shown depending on the position of the mouse on the segments of the bar. Bar segments are hyperlinks to the substance page in the database.

integrated development a specific group of substances that have undertaken to collect the data generated in the team in a commonly accessible, central database.

Before some innovative aspects of the UFT-IL database are described, the general approach taken by the author is characterized:

- Universal access by the team members to a consistent database while maintaining data security and privacy is achieved by use of a web server serving via the encrypted HTTPS protocol.
- Low maintenance costs while preserving data security are achieved by use of standard hardware[7] including an array of two mirrored hard disks (RAID 1)
- The underlying software is almost completely[8] made up from Open Source components, so no license costs have to be paid and timely fixes of security bugs can be counted on

At the time of this writing, the UFT-IL database provides information about 432 distinct chemical entities, that were received by the UFT IL team in 555 batches. Out of the 432 chemical entities, 63 were never investigated by UFT team members. They were entered into the system because of literature reports of UFT relevant properties. An acronym system was devised by Dr. R. Störmann that is used as a short notation for the substances throughout the team. As an example 1-hexyl-3-methylimidazolium tetrafluoroborate is denoted IM16 BF4.

Almost all of the chemical entities in the database are salts, composed of 154 different cations, and 59 different anions. The most comprehensive screening of the substance pool was accomplished using the IPC-81 cytotoxicity assay (348 dose-response assays) and the acetylcholinesterase inhibition assay (315 dose-response assays), followed by preliminary water solubility data (289 chemical entities), luminescence inhibition in *Vibrio fischeri* (85 dose-response assays), algal growth inhibition in *Scenedesmus vacuolatus* (45 dose-response assays), and the *Lemna minor* growth inhibition assay (41 dose- chemical entities), luminescence inhibition in *Vibrio fischeri* (85 dose-response assays), algal growth inhibition in *Scenedesmus vacuolatus* (45 dose-response assays), and the *Lemna minor* growth inhibition assay (41 dose-response assays).

The internal and external water solubility data that was collected in the database (compare Section A.14) proved to be especially useful for the UFT IL team, not only for planning bioassay experiments, but also for the team members concerned with engineering recovery methods of hydrophobic ionic liquids.

The remainder of this section is a little tour of screenshots, with the purpose of conveying an impression of the database content.

The acronym system used for naming the ionic liquids provides for a simple type of substructure search that is illustrated in Figure 3.7. It is based on the MySQL syntax for string matching, where `"IM16%"` will match any string starting with "IM16". Another example would be to search for `"% BF4"` which would find all tetrafluoroborate salts.

[7] A description of the hardware used is available at www.uft.uni-bremen.de/chemie/chem

[8] The only exception is the CACTVS chemoinformatics toolkit that is used offline for generation of chemical structure graphs as well as for calculation of molecular weights and Hill formulas. The CACTVS toolkit that is supplied by xemistry.com is closed source, but free for academic use.

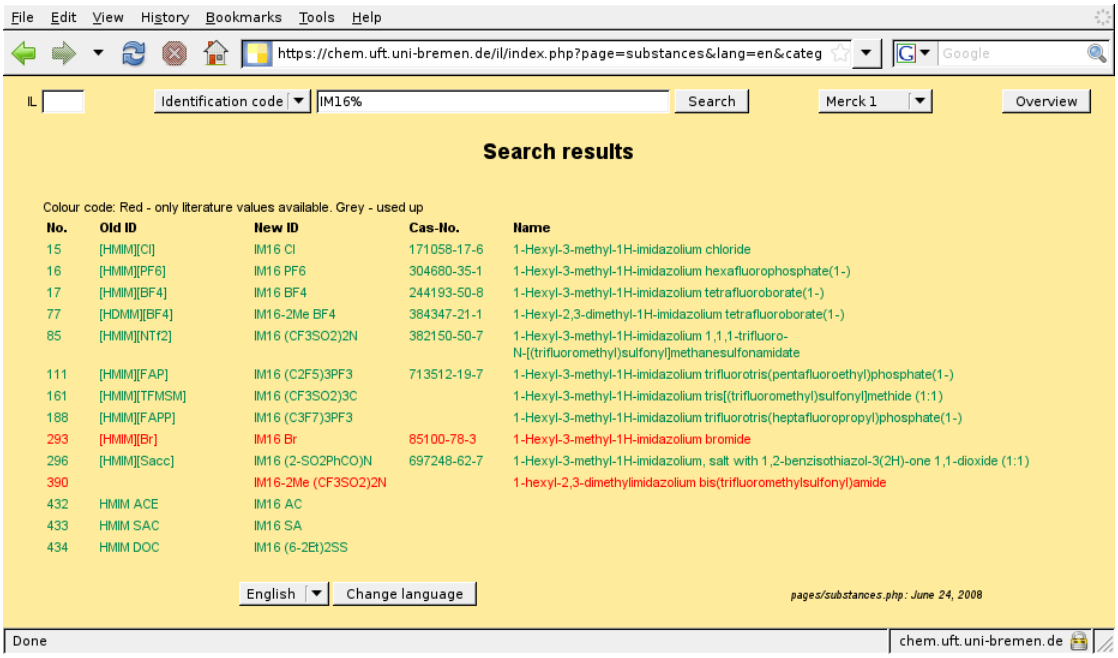


Figure 3.7: Result of a textual substructure search for the string `"IM16%"`, finding chemical entities with an acronym starting with "IM16". Chemical names according to the Chemical Abstact Service (CAS) nomenclature and CAS registry numbers were provided by Dr. R. Störmann.

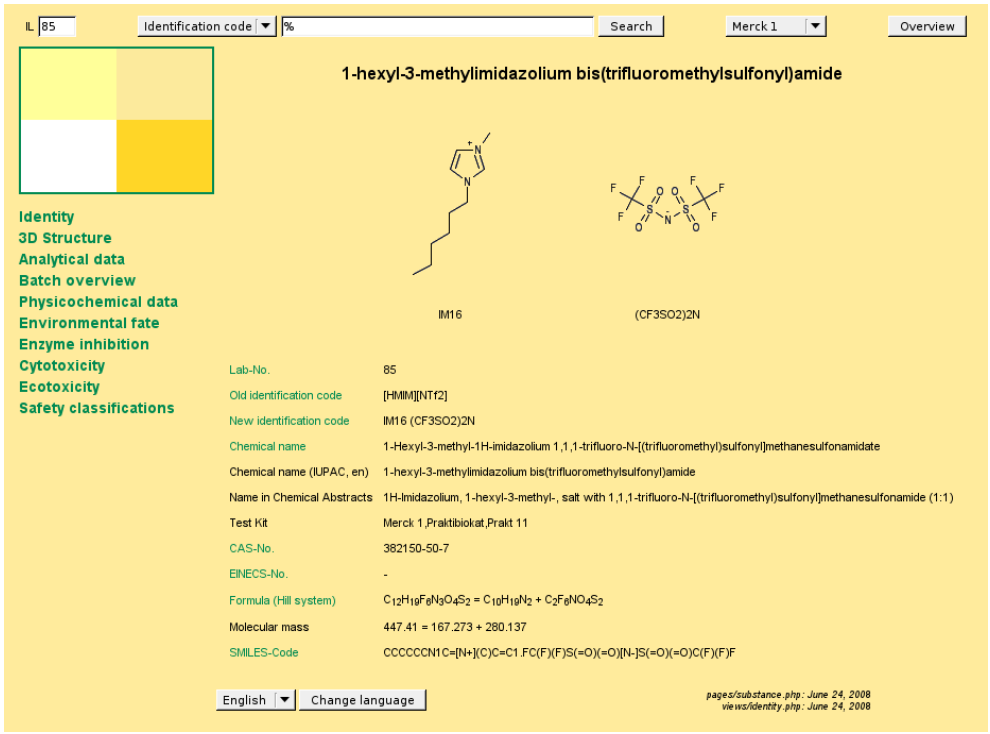
The following screenshots are presented without the menu bar, address bar and status line of the browser. Figure 3.8 a) shows information about the identity of the chemical substance with UFT number 85. Note that the two-dimensional structural formula graphs, molecular weights as well as Hill formulas for cations and anions are automatically created from the SMILES codes of cation and anion, using the chemical extensions of the Tcl scripting language provided by the CACTVS toolkit. SMILES codes for all cations and anions in the database, on which the correctness of this information is therefore based, were formulated by the author.

As can be seen in Figure 3.8 a), a chemical substance in the UFT-IL database can belong to one or several Test Kits, which are used to provide quick access to a set of substances that are relevant in a specific project.

A summary of the analytical information about IM16 (CF3SO2)2N is shown in Figure 3.8 b). The method for GC analysis used for the quantification of volatile substances is described in Section B.4. For every analysis, a chromatogram is stored in the database, so identification of contaminants can be attempted *post hoc*, if questions arise. Electrospray Ionisation (ESI) mass spectra were used for validation of the chemical structure, which helped to identify several labelling errors, although this technique does not allow for full structure elucidation.

Finally, Figure 3.9 shows a summary of the cytotoxicity data stored in the UFT-IL

a)



b)

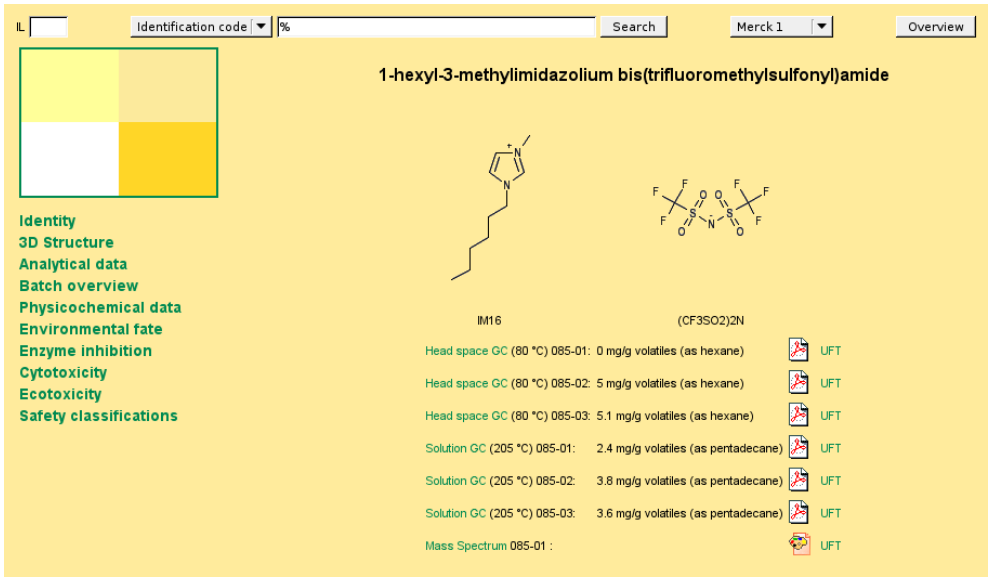
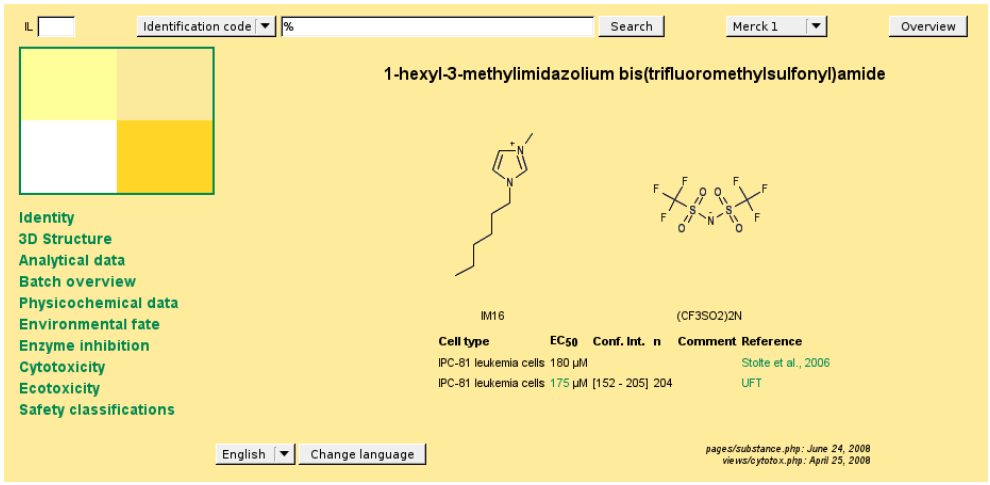


Figure 3.8: Summary pages of available identity information a) and analytical data b) about the ionic liquid with the acronym IM16 (CF3SO2)2N.

database, and an example for a dose-response curve. Bioassay data that have been published have separate entries with source attribution in the database, because sometimes additional experiments are made for the same substance, so that after a new evaluation of all the dose-reponse data using the tools described in Section 3.2, the UFT internal results may diverge from published data.

a)



b)

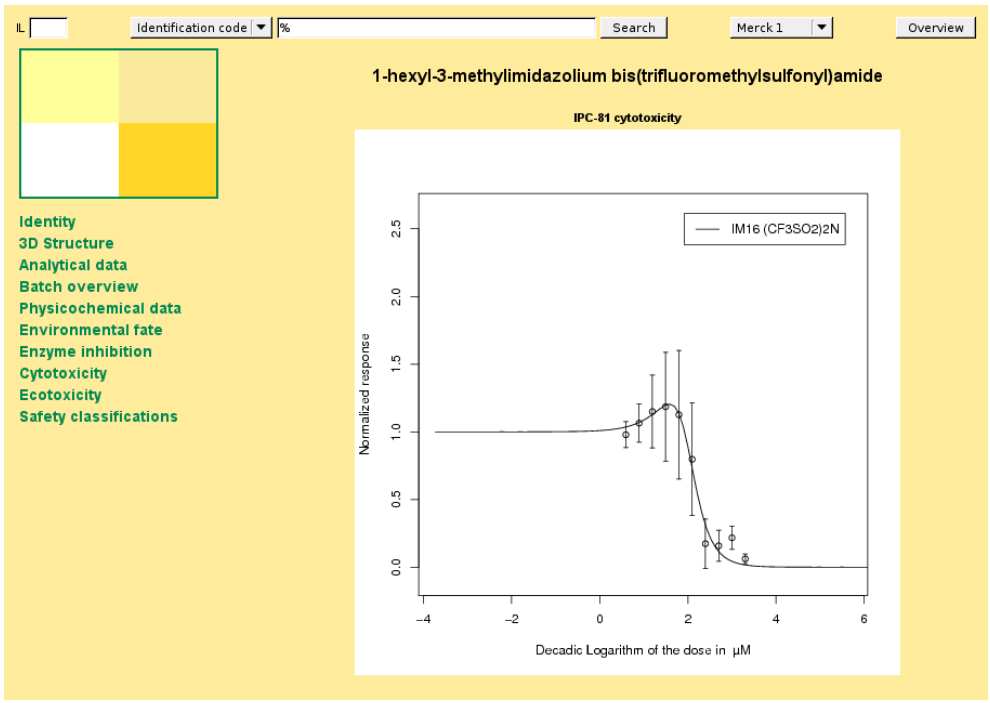


Figure 3.9: a) Summary page of cytotoxicity assay results (a) and b) dose-response curve for the ionic liquid with the acronym IM16 (CF3SO2)2N. Note that separated entries are created in the summary page for values that have been published, and the current status of the UFT internal database.

4 Advances

In this chapter, an overview of the most important findings that resulted from applying the concepts and tools described in Chapters 2 and 3 is given. For details, please refer to the original publications in Chapters A and B.

4.1 Biocides

From previous work (Ranke, 2001; Ranke and Jastorff, 2002; Böschen et al., 2004) it was known that important gaps in the risk relevant knowledge about pyrithione biocides as compared to other important antifouling biocides used in commercial shipping existed. Some progress could be made concerning calculations of pyrithione speciation and biological activities of pyrithione and related compounds (Doose et al., 2004a), but the difficulties in their chromatographic analysis (Doose et al., 2004b; Grunnet and Dahllöf, 2005) could not be solved to a satisfactory degree.

The well-known effects and analytical accessibility of Irgarol 1051 lead to its use in an attempt to establish a novel, closed multispecies system for ecotoxicological assessment (Slenzka et al., 2003). The author was involved in an attempt to establish Stir Bar Sorptive Extraction (SBSE) Thermodesorption GC/MS as a low sample volume analytical technique, especially suitable for repeatedly sampling the limited volume of water present in the test system (Behrend et al., 2006)[1].

As both lines of research did not promise to yield the desired new insights concerning the choice of more sustainable biocides, the author's team turned to investigating open questions regarding isothiazol-3-one biocides, which qualify as candidates for more sustainable biocides because of their quick disappearance from the environment under many conditions. It is immediately to be added, though, that their skin-sensitizing properties necessarily lead to restrictions regarding the use cases where their use can possibly be deemed truly sustainable.

In order to be able to make a significant contribution to the state of knowledge on isothiazol-3-one biocides, several preliminary tasks had to be fulfilled as described in Section A.11 and Section A.13.

- Selection of a representative test kit
- Purification of test compounds
- Quantification of hydrolysis rates at neutral pH

[1] The same technique was later tested as a low sample volume quantification method for organotin compounds in aqueous samples (Behrend and Ranke, unpublished results).

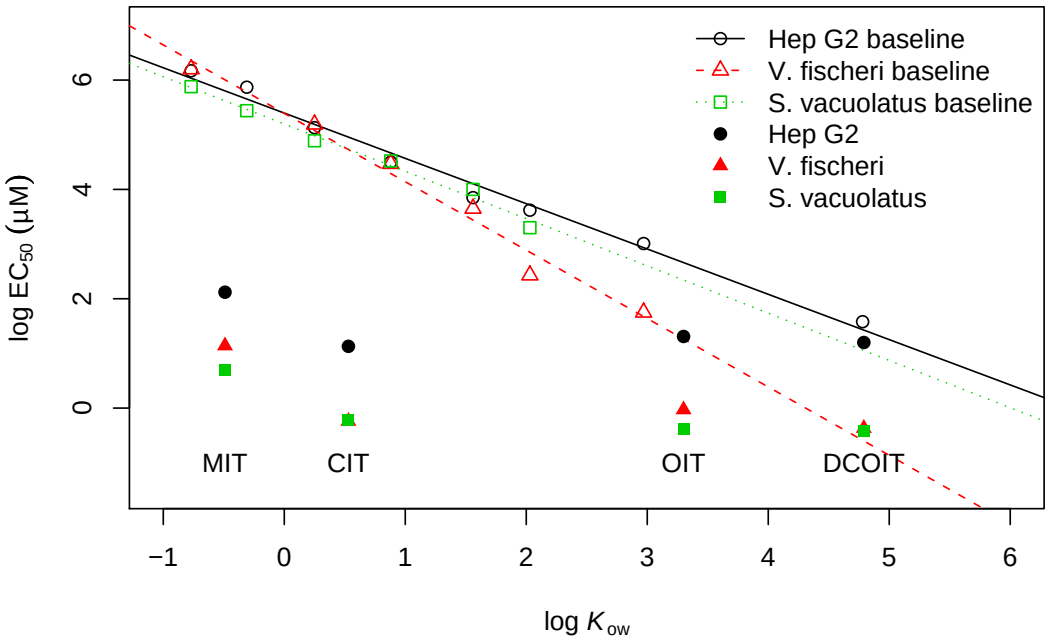


Figure 4.1: Baseline toxicity in three different cell-based bioassays established via monoalkyl alcohols (plus 2,4,5-trichlorotoluene for Hep G2 cells), and the related biological activities of the four selected isothiazol-3-one biocides. The log K_{ow} values for the biocides are derived from reversed phase HPLC.

- Quantification of reaction rates with cellular glutathione
- Determination of hydrophobicity

A synopsis of the results obtained in three different cell-based bioassays with Hep G2 cells, luminescent bacteria *Vibrio fischeri*, and monocellular green algae *Scenedesmus vacuolatus* is shown in Figure 4.1. Despite their hydrophobicities varying across more than five orders of magnitude, their varying reaction rates with glutathione (GSH), and the clear differences in their impact on the intracellular ratio of oxidized (GSSG) and reduced (GSH) glutathione, the three test compounds 5-chloro-isothiazol-3-one (CIT), *N*-octylisothiazol-3-one (OIT) and 4,5-dichloro-*N*-octylisothiazol-3-one (DCOIT) show similar effect concentrations in the three assay. Only *N*-methylisothiazol-3-one (MIT) shows higher effect concentrations in all three assays, which may be explained by its hydrophilicity.

The apparent absence of excess toxicity of DCOIT in Hep G2 cells and luminescent bacteria is especially surprising. Only in the algal assay does DCOIT show the excess

toxicity that would be expected from its fast reaction with GSH and its impact on the cellular GSH/GSSG system. For a detailed interpretation of these results, please refer to Section A.11.

An interesting possibility for interpreting the results shown in Figure 4.1 is that the isothiazol-3-ones show a structure specific basal cytotoxicity in Hep G2 cells, which is largely independent of the substitution pattern, while the other two monocellular organisms are specifically more susceptible against this compound class. This organism specific effect is in contrast to the susceptibility of the three cell types to unspecifically acting baseline toxicants, which is quite similar, with the exception of an increased sensitivity of *V. fischeri* towards the more hydrophobic monoalkyl alcohols.

4.2 Ionic liquids

At least since the NATO Advanced Research Workshop called "Green Industrial Applications of Ionic Liquids" in Crete, Greece, in April 2000, ionic liquids have been in the discussion as green (in the sense of Green Chemistry) solvents Earle and Seddon (2000). Both carefully formulated and bold claims have been and can be encountered in this discussion.

In a relatively early phase of this discussion, which has probably already reached its peak, Prof. B. Jastorff (Bremen) and Prof. B. Ondruschka (Jena) decided to collaboratively enter the field, contributing an overview of a strategy to do an integrated assessment of ionic liquids (Jastorff et al., 2003a).

To this publication, the author contributed the concept of multidimensional risk analysis, and its application to a first preliminary comparison of two ionic liquids with the commonly used solvent acetone.

The claims of greeniness of ionic liquids were mainly based on their low vapor pressure, reducing the risk of inhalative exposure as well as their flammability. Application of this concept revealed important gaps in the knowledge on risk relevant properties of ionic liquids, showing the need for the generation of almost all kinds of risk relevant data in order to provide guidance for further development of ionic liquids.

The first contribution of the author[2] to filling these gaps reproduced in Section A.1 reported the results from systematically testing the important class of 1-alkyl-3-methylimidazolium salts in two different mammalian cell lines and the luminescent bacteria *Vibrio fischeri*. In spite of the limited direct relevance of these test systems for human and ecological risk assessment, this publication received a lot of attention[3] presumably due to the early date and the relatively large number of substances tested.

[2] But see also Stock et al. (2004) where the author was involved as well

[3] In fact, of the publications providing data on ionic liquid toxicity known to the author, it has received the second most citations according to the ISI Web of Science at the time of this writing, only surmounted by Garcia et al. (2005).

4.2.1 Toxicity baselines

Because of the limitation to the methylimidazolium head group in this study, the influence of cation hydrophobicity on cytotoxicity could be described by the number of carbon atoms in the alkyl side chain, similar as in the studies of several types of low melting antimicrobial surfactants that would qualify as ionic liquids by Pernak and co-workers (2001b; 2001a, and later publications), who has been studying cationic surfactants and their antimicrobial activities since the 1970es.

The start of the strategic partnership with Merck KGaA and the generous donations of ionic liquids from them and other sources in combination with the established high content screening methods (Section 3.2) opened up the possibility to systematically test the influence of cation head groups and side chains on acetylcholinesterase inhibition (Section A.9), cytotoxicity (Section A.6) and aquatic toxicity (Section A.8). However, only the establishment of the thermodynamic hydrophobicity parameter $\log k'_0$ for the whole range of cations commonly used in ionic liquids provided the unprecedented possibility to propose baseline toxicity correlations covering also cations with relatively small hydrophobicities, for which correlations based on surface activity (Rosen, 1989; Rosen et al., 1999, 2001) are not possible, without relying on calculated $\log K_{\mathrm{ow}}$ values like the log P values used by Roberts et al. (1991; 2003a; 2003b; 2004).

Figure 4.2 shows a synopsis of the cation dependence of ionic liquid toxicity in IPC-81 cells, luminescent bacteria, monocellular green algae and duckweed. For better readability of the graph, only chloride, bromide and tetrafluoroborate salts are considered.

At first, it is noted that the effect levels of the (mostly liquid) salts of the less hydrophobic cations are similar in the cell line (48 h incubation) and the luminescent bacteria (30 min incubation), which is in accordance with the findings presented for the alkyl alcohols in Figure 4.1. In combination with the knowledge generated on the additional C6 glia cell line (Section A.1 and Section A.2) that showed similar levels of cytotoxicity, these results can be regarded to establish an approximate basal cytotoxicity level for these materials.

In analogy to the behavior of the neutral baseline toxicants in Section 4.1 the slope in the baseline toxicity for *V. fischeri* is steeper, pointing to a larger influence of hydrophobicity in this assay.

Both the 24 h algal reproduction assay and the duckweed growth inhibition assay with an exposure time of seven days are more sensitive toward the hydrophilic salts. Interestingly, the influence of hydrophobicity on *Lemna minor* is likely to be less than that on *V. fischeri*, at least if judged by the one available data point for 1-decyl-3-methylimidazolium tetrafluoroborate.

The most striking feature of Figure 4.2 however is the enormous influence of the side chain hydrophobicity of 1-alkyl-3-methylimidazolium cations on the toxicity towards green algae as originally reported in Section A.8. A thorough investigation of this remarkably strong hydrophobicity effect using various common ionic liquid head groups is highly desirable.

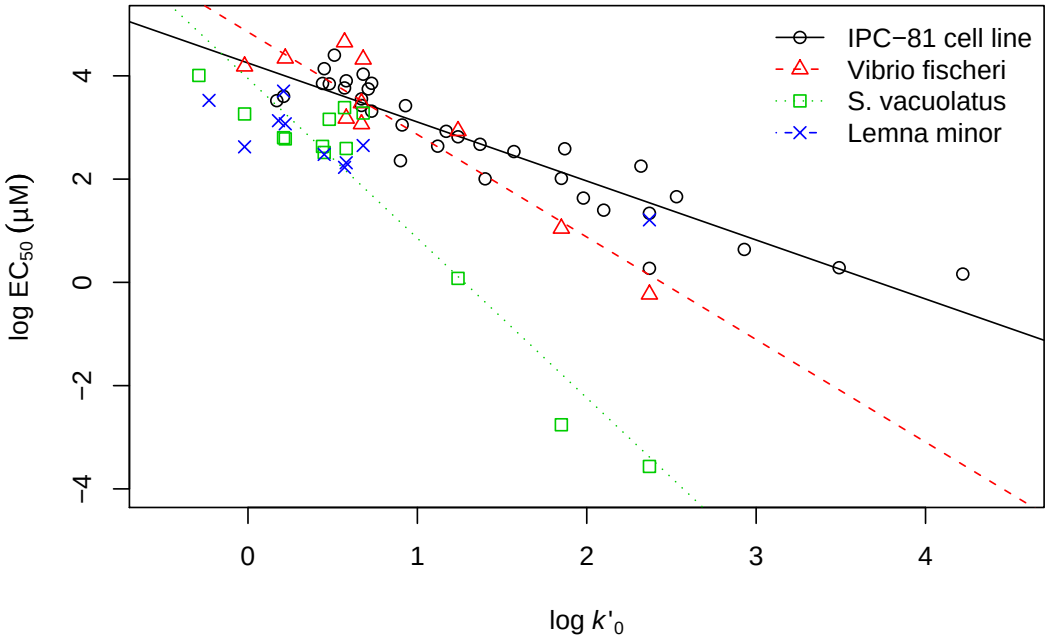


Figure 4.2: Effect concentrations in the lymphoma cell line viability, luminescence inhibition, algal growth inhibition, and duckweed growth inhibition assays in correlation with cation hydrophobicity for chloride, bromide and tetrafluoroborate salts. The two large aromatic of the quinolinium and dimethylaminopyridinium head groups were excluded.

4.2.2 Bioconcentration

One of the data gaps that were identified by careful application of the concept of ecotoxicological risk profiles to the product design of ionic liquids in Section A.7 was the lack of available data on bioconcentration and bioaccumulation of ionic liquids. In an attempt to quantify the uptake of different 1-alkyl-3-methylimidazolium ionic liquids into cells, enrichment factors in cellular material could be approximatively determined (Section A.2). It is interesting to note that the critical cellular residue of 6.8 mM that can be calculated from cellular enrichment and $\log \mathrm{EC}_{50}$ for 1-methyl-3-octylimidazolium tetrafluoroborate is within the range of 4 to 8 mM found for critical body residues of baseline toxicants in small fish (Section 2.2).

4.2.3 Anion effects

In the course of the investigations reported in Chapters A and B, the effect of the anion on the toxicity of ionic liquids in cells and other organisms has been discussed in detail.

In Section A.1, no consistent pattern of anion influence could be found. This finding has then been challenged by several authors, but also by the author and coworkers himself, in the most comprehensive study of anion effects on ionic liquid toxicity to date (Section A.3). In this study, an anion effect ratio (AR) was proposed for the quantification of this anion effect, but in the same publication, the hypothesis of concentration addition (CA) between cation and anion was tested.

The CA approach predicts that the anion effect ratio will be smaller, the more hydrophobic and therefore toxic the cation, which seemed to be the case, based on the data at hand, especially for the bis(trifluoromethylsulfonyl)amide salts.

In order to clarify the understanding of the anion effect, Figure 4.3 presents ionic liquid cytotoxicity data of 1-alkyl-3-methylimidazolium salts in the IPC-81 cell line in a similar way as in Section A.3, but for a reduced set of anions. In order to test the hypothesis of a constant anion effect ratio across varying cation hydrophobicities, a trend line is drawn for each anion, resulting from a linear regression of cytotoxicity against hydrophobicity.

Several interesting conclusions can be drawn from Figure 4.3. First of all, a large variation of the cytotoxicities, quantified by their $\log \mathrm{EC}_{50}$ values around the respective trend lines is obvious for most anions. Secondly, the slopes of the trend lines in Figure 4.3 are not equal. In particular, the slope off the trend line for the bis(trifluoromethylsulfonyl)amide could suggest that the toxicity increasing effect of this anion decreases with increasing cation hydrophobicity. This explains why the tentative hypothesis of similar mode of action of cation and anion according to concentration addition was tenable in the original publication.

On the other hand, regression lines in Figure 4.4 have approximately the same slope, which suggests to formulate a model with constant anion effect ratios, in mathematical analogy to the water solubility model proposed in Section A.14. It must to be noted, that the data used for the regressions in Figure 4.4 were cropped to data pairs with $\log k'_0 > 0.3$, in order to avoid a bias in regression slopes, that would otherwise resulting

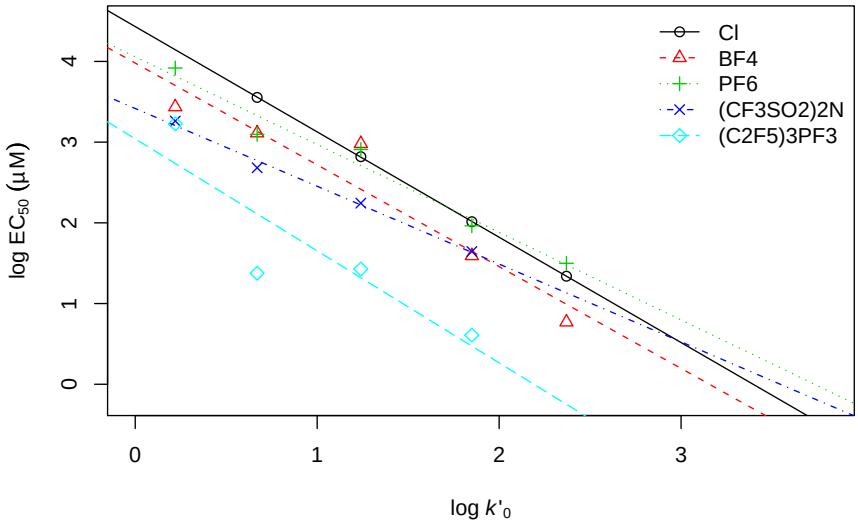


Figure 4.3: Cytotoxicity in the IPC-81 cell line plotted against cation hydrophobicity for 1-alkyl-3-methylimidazolium salts of five selected anions.

from the arbitrary upper limit of the effect concentrations chosen for the determination of $\log \mathrm{EC}_{50}$ values in this assay.

Also, in both Figures, only ionic liquids with cations less hydrophobic than $\log k'_0 = 4$ are considered, in order to avoid hitting the solubility cutoff described in Section A.5.

Considering the large differences in water solubility and even in water miscibility of chlorides, tetrafluoroborates and hexafluorophosphates, it is surprising that their anion effect, if significant at all, is only small. Comparison of the distances between the regression lines in Figure 4.4 with the analogous differences reported in Section A.14 for the water solubility model clearly supports the conclusion that the anion influence on water solubility is much greater than the anion influence on cytotoxicity. Thus, ionic liquid water solubility is not a quantitative predictor of cytotoxicity. In fact, the more hydrophobic hexafluorophosphates even seem to be generally less cytotoxic than the tetrafluoroborates.

A possible explanation for the small influence of anion hydrophobicity on cytotoxicity — with the exception of the very hydrophobic trifluorotris(pentafluoroethyl)phosphate anion — could be that the anions that the cations have to take with them to their site of action because of electroneutrality are only to a minor part the counteranions of the tested ionic liquid.

A more political implication of the authors' findings is the observation that the fate of ionic liquid ions in aquatic environments with a sufficient ionic strength will likely

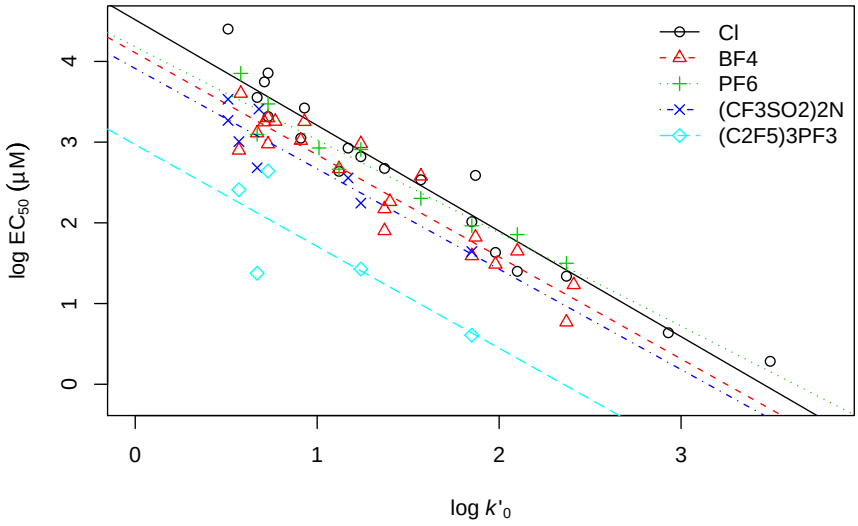


Figure 4.4: Cytotoxicity in the IPC-81 cell line plotted against cation hydrophobicity for 1-alkyl-3-methylimidazolium for all available salts of five selected anions, except for the quinolinium and dimethylaminopyridinium compounds, and limited to the log k'_0 range between 0.3 and 4.

be separate. Therefore, it was suggested in Section A.7 that the risks of cations and anions to such environments should be assessed separately. While this would make the assessment strategy for a specific ionic liquid dependent on the type of the exposed environment, as suggested in Section B.3, it would also reduce the amount of testing required for assessing ionic liquids sharing a common cation and anion for the affected endpoints.

Nevertheless, the problem of the blindness of the common single substance risk assessment for combinatorial effects occurring in the environment remains. Even though the concept of concentration addition for cation and anion effects is not tenable after the above analysis, uptake and biological action of ionic liquid cations or anions from environmental media will depend on the counterions present in them.

5 Outlook

In the above, an attempt has been presented at improving the tool set available for in turn improving the sustainability of chemical products and processes. Preference was given to flexible risk assessment and testing strategies that can be adapted to the specific situation, while generality was sought in the choice of scientific concepts, aiming at possibilities to reduce the number of variables with which complex situations can be described.

Both aspects require responsible scientists, using the flexibility for gaining realistic insights instead of trying to generate evidence for preconceived results, and taking advantage of generalizations, while respecting that it will not be possible to completely predict or even describe a complex world with simple concepts.

One common aspect of the research areas described above is their use of data, generated in house, obtained from scientific literature, or obtained from third party databases. Traditionally, in science, great efforts are directed at the improvement of scientific knowledge. Especially when dealing with a large number of different materials, the improvement of the data base of the scientific community, regarding quality, availability, reliability and convenience should receive much more attention.

A prominent approach to improve the availability and usability of scientific data is the Blue Obelisk (blueobelisk.sourceforge.net), a "group of chemists/programmers/-informaticians" who advocate the principles of Open Data, Open Standards, Open Source (ODOSOS), but not necessarily of Open Access.

Regarding Open Data and Open Standards, the present thesis is more of a counterexample, since the database used in the process of ionic liquid product development as described in Section 3.3.2 is only accessible for team members, and the format in which they are stored is not publicly specified, except for some hints on the database layout given in Section A.4.

The author has long been aware of these shortcomings, and only recently it was agreed between Merck KGaA and the UFT ionic liquids team — upon initiative of the author — to create a publicly accessible database, containing at first the data already published on ionic liquids in peer reviewed publications in an easily accessible manner, and in a second stage also data that has been generated in the course of later projects which have not been published in the traditional way yet.

In order to be able for other scientists to use that data without having to manually access a large number of web pages and to copy numbers from a web browser to their own data systems, it has been proposed by the author at the GDCh conference of the section of environmental chemistry and ecotoxicology (Ranke, 2007b) to establish ecotoxicological dialects of the eXtensible Markup language (XML), similar, but again more flexible than the XML dialects that have been established for 102 endpoints as

part of the IUCLID system for exchanging risk relevant data with the newly founded European Chemicals Agency[1].

Clearly, the IUCLID system has been devised with the requirements of the REACH regulation in mind, which, among other purposes, also tries to generate the database for improved structure-activity relationships as proposed in this thesis. It remains to be seen, if the scientific community will be able to use these tools, if it will be necessary to adapt them, or if it will be advantageous to create solutions (data standards, data exchange tools and data analysis tools) that are wholly owned by the scientific community.

Comparative risk analysis of products or processes is a demanding and time-consuming endeavour, and at the end, not only the information is associated with uncertainty, but also the consensus that can be reached.

Sustainable Development in general and Sustainable Chemistry in particular are common challenges for the different subsystems of society, requiring to overcome the communication barriers between science, economy, jurisdiction and others in order to establish a common sense of sustainability.

[1] More information can be found on the EChA website (echa.europa.eu) and the description of the IUCLID XML format at the Joint Research Centre (JRC) ecbw-biu5.jrc.it/index.php?fuseaction=home.format&type=public

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A Core publications

A.1 Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1 cell viability assays

Johannes Ranke, Kerstin Mölter, Frauke Stock, Ulrike Bottin-Weber, Johanna Poczobutt, Jens Hoffmann, Bernd Ondruschka, Juliane Filser, and Bernd Jastorff

Ecotoxicology and Environmental Safety **58** (3) 396-404

Corrigendum: *Ecotoxicology and Environmental Safety* **60** (3) 350

Detailed biological studies of methyl- and some ethylimidazolium ionic liquids in luminescent bacteria as well as in the IPC-81 (leukemia cells) and C6 (glioma cells) rat cell lines are presented. Effective concentrations in these test systems are generally some orders of magnitude lower than effective concentrations of the conventional solvents acetone, acetonitrile, methanol, and methyl *t*-butyl ether. No general influence of the anionic compound in the ionic liquids on toxicity could be found, although they seem to modulate toxicity in some cases. The clear influence of the alkyl chain length on toxicity was quantified by linear regression analysis. Alkyl chain length of the longer alkyl chain was varied from 3 to 10 carbon atoms. Consequences for a design of sustainable alternative solvents are briefly sketched.

doi: 10.1016/S0147-6513(03)00105-2

A.2 Sorption, cellular distribution and toxicity of imidazolium ionic liquids in mammalian cells — Influence of lipophilicity

Johannes Ranke, Monika Cox, Anja Müller, Christiane Schmidt, and Detmar Beyersmann

Toxicological and Environmental Chemistry **88** (2) 273-285

13 pages

doi: 10.1080/02772240600589505

A.3 Anion effects on the cytotoxicity of ionic liquids

Stefan Stolte*, Jürgen Arning, Ulrike Bottin-Weber, Frauke Stock, Karen Thiele, Mark Uerdingen, Urs Welz-Biermann, Bernd Jastorff, and Johannes Ranke

Green Chemistry **8** (7) 621-629

Most recent investigations concerning the toxicological and ecotoxicological risk potentials of ionic liquids are predominantly focusing on the cation moieties. In this study we elucidate, whether the anion species commonly used in ionic liquids are exhibiting intrinsic cytotoxic effects and if these effects can be rationalised by thinking in terms of structure-activity relationships (T-SAR). As test system to measure the cell viability as toxicologically relevant endpoint the IPC-81 rat leukemia cell line and the WST-1 assay were employed. Our results show an anion effect in ionic liquids on cytotoxicity for 10 of 27 tested anions. For the remaining 17 anions from our test kit no significant effect was found. With respect to structure-activity relationships, lipophilicity and/or vulnerability to hydrolytic cleavage seem to be the key structural features leading to the observed anion cytotoxicity. We also conclude that the model of concentration addition may be useful to estimate the EC50 values of ionic liquids that have not been tested or even synthesised yet. This can help to design not only task specific but also inherently safer ionic liquids.

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A.4 Fitting dose-response curves from bioassays and toxicity testing

Johannes Ranke*

R News **2006** (3) 7-12

Full paper reprinted here

Appendix

The data set used for this article is shown in the table. It is taken from Deming and Turoff (1978) where it was published in retention times. For this article, the data has been converted to their corresponding capacity factors.

pH	BA	OABA	PABA	HOBA
3.79	34.21	15.06	8.85	14.30
3.79	34.27	14.64	8.33	14.52
4.14	25.85	14.24	8.00	12.30
4.38	20.46	13.33	7.58	10.76
4.57	15.61	12.61	6.82	8.91
4.74	12.42	11.33	5.76	7.24
4.74	11.42	10.55	5.76	7.06
4.92	9.64	10.15	5.09	5.94
5.11	7.30	9.12	4.15	4.52
5.35	5.15	6.36	2.88	3.09
5.67	3.18	3.92	1.60	1.68
5.67	3.18	3.92	1.58	1.62

Fitting dose-response curves from bioassays and toxicity testing

by Johannes Ranke

Introduction

During the development of new chemicals, but also in risk assessment of existing chemicals, they have to be characterized concerning their potential to harm biological organisms. Characterizing chemicals according to this potential has many facettes and requires various types of experiments. One of the most important types is the dose-response experiment.

In such experiments, the responses of biological organisms to different doses[1] are observed in a quantitative way. Examples of the observed variables (endpoints of toxicity) are the length of wheat seedlings after being exposed to different concentrations of the chemical substance for a defined time interval, the activity of luminescent bacteria, the ability of cell cultures to reduce a specific dye, the growth rate according to number of individuals or biomass, the number of viable offspring and many others.

These observed variables have in common that a reference magnitude for healthy and viable organisms can be defined (normalised response level $r = 1$), and that the magnitude of the variable (response) is limited by a zero response ($r = 0$) where the maximum of the effect is observed. The **drfit** package covers the case where there is a continuum of possible response values between 0 and 1 (inclusive). Additionally, responses above 1 are frequently observed due to variability or as the result of stimulation by a subtoxic dose, and even responses below 0 may be present, depending on the type of data and the applied preprocessing.

If the responses are binomial, such as life and death for a number of individuals, it is adviseable to choose the readily available `glm` fitting procedures (generalized linear models), where the probit and logit links are already built-in (e.g. Chapter 7.2 in Venables and Ripley (2002)) or to look into the **drc** package.

Dose-response relationships for continuous response tests can generally be expressed as

$$r = f(d, \vec{p}) + \epsilon \quad (1)$$

where r is the normalised response at dose d, $f(d, \vec{p})$ is the model function with parameter vector $\vec{p}$, and ϵ is the error variable describing the variability in the observations not explainable by the model function $f(d, \vec{p})$.

This article shows how different model functions $f(d, \vec{p})$ can be conveniently fitted to such dose-response data using the R package **drfit**, yielding the vector of parameters $\vec{p}$ that gives the description of the data with the least residual error. The fitting can be carried out for many substances with a single call to the main function `drfit`.

The results that the user will probably be most interested in are the doses at which a response of 50 % relative to healthy control organisms is to be expected (termed ED_{50}), as this is a very robust parameter describing the toxicity of the substance toward the organism investigated.

The **drfit** package internally uses the R function `nls` for nonlinear regression analysis as detailed by Bates and Watts (1988). Confidence intervals for the model parameters are calculated by the `confint.nls` function from the **MASS** package as described in Venables and Ripley (2002).

drfit defines a dose-response data representation as a special case of an R dataframe, facilitates fitting standard dose-response models (probit, logit,

[1] The term dose is used here in a generalised way, referring to doses in the strict sense like mg oral intake per kg body weight as well as to measured concentrations in aquatic toxicity tests or nominal concentrations in cell culture assays.

weibull and linlogit at the time of this writing), and a function to produce different types of plots of the data as well as the fitted curves.

Optionally, the raw data can be kept in an external database connected by **RODBC**. This has proven to be useful if the data of a large number of dose-response experiments have to be evaluated, as for example in bioassays based on microtiter plates.

Recently, the R package **drc** containing similar functionalities to **drfit** has been uploaded to CRAN. Unfortunately, I have noticed the existence of this package only during the preparation of this article, after having maintained **drfit** on CRAN for almost one and a half years. Maybe in the future it will be possible to join forces.

In this introductory article, it is explained how the input data must be formatted, how dose-response curves are fitted to the data using the `drfit` function and in what ways the data and the models can be plotted by the `drplot` function. Since the package was actively developed during the preparation of this article, the reader is advised to upgrade to the latest `drfit` version available. Note that R $\geq$ 2.1.0 is needed for recent `drfit` versions.

Collecting dose-response data

The `drfit` function expects the dose-response data as a data frame containing at least a factor called 'substance', a vector called 'unit' containing the unit used for the dose, a column 'response' with the response values of the test system normalized using the "natural" zero response as 0, and the response of the control organisms as a "natural" 1. Therefore, values outside this interval, and especially values above 1 may occur in the normalized data. An example of such data can be easily obtained from the built-in dataset `XY`.

```
> library(drfit)
> data(XY)
> print(XY,digits=2)
   nr.    substance dose unit fronds response
1    1      Control    0 mg/L    174     1.050
2    2      Control    0 mg/L    143     0.973
3    3      Control    0 mg/L    143     0.973
4    4 Substance X   10 mg/L    147     0.983
5    5 Substance X   10 mg/L    148     0.986
6    6 Substance X   10 mg/L    148     0.986
7    7 Substance X  100 mg/L     63     0.651
8    8 Substance X  100 mg/L     65     0.663
9    9 Substance X  100 mg/L     55     0.598
10  10 Substance X  300 mg/L     20     0.201
11  11 Substance X  300 mg/L     22     0.238
12  12 Substance X  300 mg/L     25     0.288
13  13 Substance X 1000 mg/L     13     0.031
14  14 Substance X 1000 mg/L     16     0.113
15  15 Substance X 1000 mg/L     16     0.113
16  16      Control    0 mg/L    153     0.999
17  17      Control    0 mg/L    144     0.975
18  18      Control    0 mg/L    163     1.024
19  19 Substance Y   10 mg/L     20     0.201
20  20 Substance Y   10 mg/L     19     0.180
21  21 Substance Y   10 mg/L     21     0.220
22  22 Substance Y  100 mg/L     13     0.031
23  23 Substance Y  100 mg/L     12     0.000
24  24 Substance Y  100 mg/L     13     0.031
25  25 Substance Y  300 mg/L     12     0.000
26  26 Substance Y  300 mg/L     12     0.000
27  27 Substance Y  300 mg/L     14     0.061
28  28 Substance Y 1000 mg/L     12     0.000
29  29 Substance Y 1000 mg/L     12     0.000
30  30 Substance Y 1000 mg/L     12     0.000
```

Normalisation of the response data is not done within the **drfit** package. It can either be carried out with a typical spreadsheet file, with some extra lines of R code, or by an external procedure, while or before the data is read into a database.

If the data is collected and normalised using MS Excel, it can be easily transferred to R by saving it in CSV format, and reading it in using the R function `read.csv2` or alternatively by the `read.xls` function from the **gdata** package. If OpenOffice.org Calc is being used, and the default values are used for exporting the data in CSV format, the function `read.csv` is very helpful.

Figure 1 shows a possible spreadsheet layout for capturing dose-response data including both the observed endpoint (number of fronds in this case) and the normalized response values.

Total growth inhibition is in this case the natural lower limit of the response and the response will therefore be zero if the number of duckweed (*Lemna minor*) fronds stays at the initial level n_0 during the observation time. The natural reference for the healthy organisms (response=1) is in this case given by the growth rate of the controls μ_c, calculated by

$$\mu_c = \frac{\ln(\bar{n_c}) - \ln(n_0)}{t - t_0} \tag{2}$$

where $\bar{n_c}$ is the mean number of fronds in the control experiments after the observation time. The growth rates μ_i are calculated in the same way, and the normalized responses are then easily obtained by

$$r_i = \frac{\mu_i}{\mu_c} \tag{3}$$

If the spreadsheet from Figure 1 (which can be found at http://www.uft.uni-bremen.de/chemie/ranke/data/drfit/) were exported by writing a CSV file, this file could be processed by something like

```
> d <- read.csv('sampledata.csv',skip=2,dec=',')
```

depending on the path to the CSV file, the number of lines before the column headings and the decimal separator used.

F4 =(LN(E4)-LN(12))

	A	B	C	D	E	F
1	Concentration-response data for the Lemna growth tes					
2						
3	**nr.**	**substance**	**dose**	**unit**	**fronds**	**response**
4	1	Control	0	mg/L	174	1,0496
5	2	Control	0	mg/L	143	0,9726
6	3	Control	0	mg/L	143	0,9726
7	4	Substance X	10	mg/L	147	0,9834
8	5	Substance X	10	mg/L	148	0,9861
9	6	Substance X	10	mg/L	148	0,9861
10	7	Substance X	100	mg/L	63	0,6509
11	8	Substance X	100	mg/L	65	0,6631
12	9	Substance X	100	mg/L	55	0,5976
13	10	Substance X	300	mg/L	20	0,2005
14	11	Substance X	300	mg/L	22	0,2379
15	12	Substance X	300	mg/L	25	0,2881
16	13	Substance X	1000	mg/L	13	0,0314
17	14	Substance X	1000	mg/L	16	0,1129
18	15	Substance X	1000	mg/L	16	0,1129
19	16	Control	0	mg/L	153	0,9991
20	17	Control	0	mg/L	144	0,9754
21	18	Control	0	mg/L	163	1,0240
22	19	Substance Y	10	mg/L	20	0,2005
23	20	Substance Y	10	mg/L	19	0,1804
24	21	Substance Y	10	mg/L	21	0,2197
25	22	Substance Y	100	mg/L	13	0,0314
26	23	Substance Y	100	mg/L	12	0,0000
27	24	Substance Y	100	mg/L	13	0,0314
28	25	Substance Y	300	mg/L	12	0,0000

Figure 1: Data structure for a typical toxicity test in OpenOffice Calc. Note that the response column is calculated (see text).

Fitting and plotting

A quick result for a compatible dataframe can usually be obtained by a simple call to `drfit`

```
> rXY <- drfit(XY)
```

The contents of the dataframe `rXY` containing the results of the fitting procedure are shown in Figure 2. Each fitted dose-response model (usually only one per substance) produces one line. The number of dose levels `ndl` is reported, the total number of data points used for the model fitting `n`, the decadic logarithms of the lowest dose `lld` and the highest dose `lhd` tested.

The next column contains the type of the dose-response model fitted (probit, logit, weibull or linlogit) or, if not applicable, a classification of the substance data as "active" (if the response at the lowest dose is < 0.5) , "inactive" (if the response at the highest dose is > 0.5) or "no fit".

The log ED_{50} is given with its confidence interval as calculated by the `confint.nls` function from the **MASS** package. This only works if the log ED_{50} is one of the model parameters. Therefore, in the case of the weibull model, no confidence interval is given.

Finally, the residual sum of squares `sigma` is listed and the fitted parameters `a` and `b`, or, in the case of the three paramater model `linlogit`, the parameters `a`, `b` and `c` are listed.

Once the `drfit` function has been successfully called and the result assigned a name (`rXY` in this case), dose-response plots for the fitted data can easily be created using the `drplot` function. The following example produces a single graph (`overlay=TRUE`) with the fitted dose-response curves and raw data (`dtype="raw"`) for all substances and fitted models in dataframes `XY` and `rXY` using color (`bw=FALSE`). Additionally, the scatter of the responses in control experiments can be displayed, by setting the argument `ctype` to `"std"` or `"conf"`: as shown in Figure 3.

```
> drplot(rXY,XY,overlay=TRUE,bw=FALSE,
ylim=c("auto",1.3),dtype="raw", ctype="conf")
```

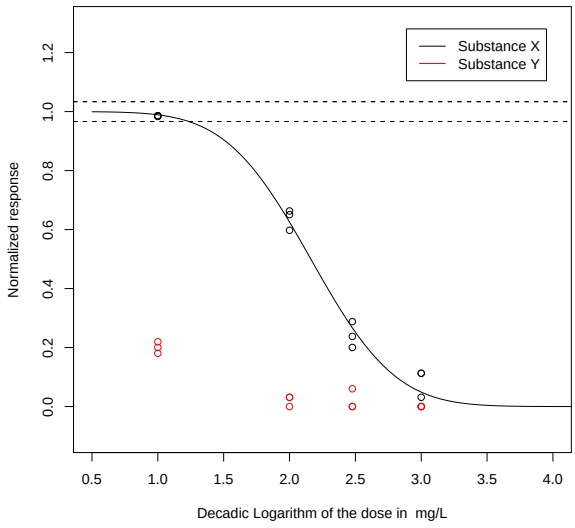

Figure 3: Output of the `drplot` function for the sample data `XY` from the package.

If the user prefers to view the raw data with error bars, the argument `dtype` can be set to `"std"` for showing standard deviations (default) or `"conf"` for showing confidence intervals.

In the following, the analysis of a somewhat more complicated, but also more interesting example is illustrated, which has been published by Ranke et al. (2004) before the basic `drfit` code was packaged .

First, dose-response curves with the default settings of `drfit` are generated as shown in Figure 4.

```
> data(IM1xIPC81)
> dIM <- IM1xIPC81
> rIM <- drfit(dIM)
> drplot(rIM,dIM,overlay=TRUE,bw=FALSE)
```

```
> print(rXY,digits=2)
    Substance ndl  n  lld  lhd    mtype logED50 2.5% 97.5% unit sigma   a    b
1     Control   1  6 -Inf -Inf inactive      NA   NA    NA mg/L    NA  NA   NA
2 Substance X   4 12    1    3   probit     2.2  2.1   2.2 mg/L 0.041 2.2 0.51
3 Substance Y   4 12    1    3   active      NA   NA    NA mg/L    NA  NA   NA
```

Figure 2: Contents of the dataframe containing the results from the fitting procedure for example data from the package (see text for explanations).

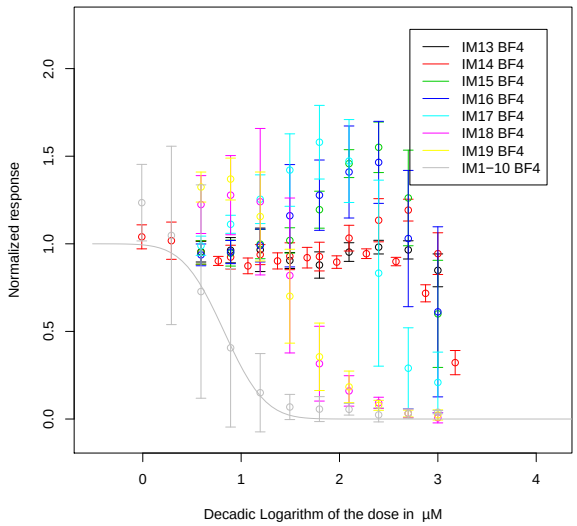

Figure 4: Dose-response plot showing the toxicities in a homologous series of compounds and the fitted probit model for IM1-10 BF4.

The graph shows that only one dose-response curve is fitted with the built-in default arguments of the `drfit` function and that the legend is interfering with the data. It is obvious that for almost all substances in this data, response values > 1 are caused in a certain dose range, a phenomenon which is called hormesis. In order to properly model such data, the so-called linear-logistic dose-response model has been introduced by Brain and Cousens (1989). The `drfit` package makes use of it in the parameterization suggested by van Ewijk and Hoekstra (1993), which allows for a convenient calculation of confidence intervals of the ED_{50}.

To include the linear-logistic model (`linlogit` in `drfit` terminology) in the fitting procedure and list the results including confidence intervals for a confidence level of 90 % two-sided, one simply calls

```
> rIM2 <- drfit(dIM,linlogit=TRUE,level=0.9,
chooseone=FALSE)
```

First, the `linlogit` argument causes the linlogit model to be additionally tried. Then, the argument `chooseone=FALSE` leads to reporting one line for each fitted model. If the argument `chooseone` is set to `TRUE` (default), only the first convergent dose-response model (probit and linlogit in this case) from the somewhat arbitrary sequence linlogit > probit > logit > weibull is reported.

The dataframe with the results shown in Figure 5 accordingly lists all instances of fitted models, and gives confidence intervals for the log ED_{50} values.

Then, a customized plot can be generated:

```
> drplot(rIM2,dIM,overlay=TRUE,bw=FALSE,
xlim=c("auto",5))
```

The `xlim` argument to `drplot` fixes the interference between legend and data. Furthermore, the plot produced in the above example shown in Figure 6 shows two fitted dose-response curves for the substance IM1-10 BF4 (grey lines), one for the probit and one for the linlogit model.

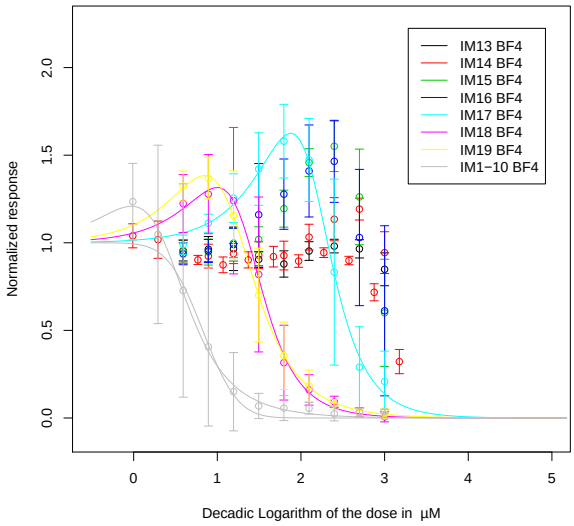

Figure 6: Dose-response plot showing the dose-response curves for a homologous series of compounds and all fitted linlogit and probit models.

External databases

Certain screening bioassays can be carried out with relatively low investments of time and money, so

```
> print(rIM2,digits=2)
   Substance ndl   n    lld lhd    mtype logED50   5%  95%   unit sigma    a    b     c
1   IM13 BF4   9  81  0.592 3.0 inactive      NA   NA   NA microM    NA   NA   NA    NA
2   IM14 BF4  20 216 -0.010 3.2   no fit      NA   NA   NA microM    NA   NA   NA    NA
3   IM15 BF4   9 135  0.592 3.0 inactive      NA   NA   NA microM    NA   NA   NA    NA
4   IM16 BF4   9 108  0.592 3.0 inactive      NA   NA   NA microM    NA   NA   NA    NA
5   IM17 BF4   9  81  0.592 3.0 linlogit    2.58 2.52 2.65 microM  0.24 2.58 2.30 0.015
6   IM18 BF4   9 135  0.592 3.0 linlogit    1.68 1.63 1.73 microM  0.23 1.68 2.24 0.057
7   IM19 BF4   9  81  0.592 3.0 linlogit    1.65 1.61 1.69 microM  0.15 1.65 1.98 0.110
8 IM1-10 BF4  11 162 -0.010 3.0 linlogit    0.77 0.70 0.84 microM  0.30 0.77 1.94 0.458
9 IM1-10 BF4  11 162 -0.010 3.0   probit    0.83 0.75 0.90 microM  0.31 0.83 0.33    NA
```

Figure 5: Contents of the dataframe containing the results from the fitting procedure for the chain length data `IM1xIPC81` from the package (see text for explanations).

large volumes of dose-response data can build up (high-throughput screening/high-content screening). The drfit package makes it possible to retrieve data stored in databases accessible by ODBC using the RODBC package internally. Since RODBC works on Windows, Mac OS X and Unix platforms, the code is platform- and database independent to a high degree.

For storing cytotoxicity data in a MySQL database, the following minimum database definition is advisable:

```
CREATE TABLE `cytotox` (
  `pk` int(11) unsigned NOT NULL auto_increment,
  `plate` int(11) NOT NULL default '0',
  `experimentator` varchar(40) NOT NULL
    default '',
  `substance` varchar(100) NOT NULL default '',
  `celltype` varchar(20) NOT NULL default '',
  `conc` float NOT NULL default '0',
  `unit` set('unit1','...') default 'unit1',
  `viability` float NOT NULL default '0',
  `performed` date NOT NULL
    default '0000-00-00',
  `ok` set('not ok','ok','?','no fit')
    default '?',
  PRIMARY KEY  (`pk`),
)
```

The `pk` and the `performed` data field are not interpreted by the package, databases with any other columns missing might work but have not been tested.

The column called `viability` contains the normalised response that has been calculated at the time of the data import into the database. Of course, the Data Source has to be configured to be a valid and accessible ODBC DSN on the platform used, e.g. by installing and configuring unixodbc and myodbc under Linux or MyODBC under Windows. This also involves setting up the MySQL server to listen to network connections, if it is not located on the local computer, and adequate MySQL user privileges.

With such a setup, the `drdata` function from the package can be used to conveniently retrieve data from the database and evaluate it with the `drfit` and `drplot` functions:

```
> s <- c("Sea-Nine","TBT","ZnPT2")
> d <- drdata(s,experimentator = "fstock",
whereClause="performed < 2006-04-04")
> r <- drfit(d,linlogit=TRUE)
> drplot(r,d,dtype="none",
bw=FALSE,overlay=TRUE)
```

The `whereClause` argument to the `drdata` function allows for flexible selection of data to be used for the analysis, e.g. by using comparison operators on columns containing dates as illustrated in the above example.

Additionally, the use of the argument `dtype="none"` to the `drplot` function is shown, which leads to the display of the fitted models only, without the data, as shown in Figure 7.

In the UFT Center of Environmental Research and Technology, we use the `drfit` package for regular batch-processing of all our dose-response data from several bioassays for a substance library of more than 200 compounds. The results are in turn written to a database, and the `drplot` function is used to create updated dose-response plots every time the raw data has been augmented. The whole process of fitting all data and producing the plots takes less about 1 minute on an 1600 MHz AMD Sempron PC for the cytotoxicity data for 227 substances, provided that the new data has been checked by the `checkplate` and `checksubstance` functions, which allow for an easy validation of experimental dose-response data generated by plate-reader bioassays stored in a `drfit` conformant MySQL database.

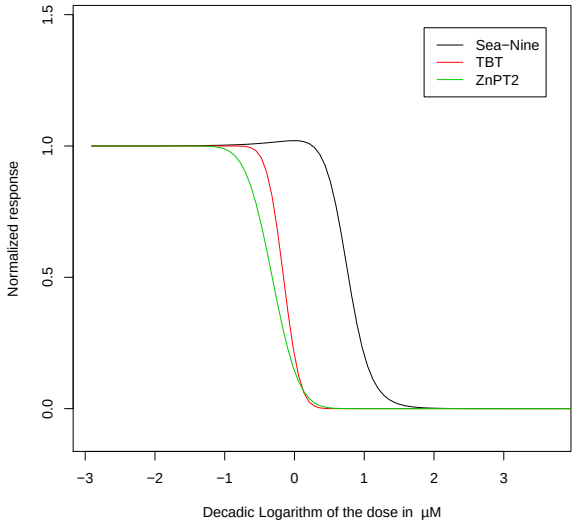

Figure 7: Dose-response plot showing the fitted dose-response curves for three antifouling biocides in the cytotoxicity assay fitted with the linlogit model.

The whole system provides the basis for analysis of the toxicity data, e.g. by (Quantitative) Structure-Activity Relationships (SAR/QSAR), which may provide a deeper chemical understanding of the interaction of the chemicals with biological organisms.

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P. H. van Ewijk and J. A. Hoekstra. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. *Ecotoxicology and Environmental Safety*, 25:25–32, 1993. 10

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The pls package

by Bjørn-Helge Mevik

Introduction

The **pls** package implements *Partial Least Squares Regression* (PLSR) and *Principal Component Regression* (PCR). It is written by Ron Wehrens and Bjørn-Helge Mevik.

PCR is probably well-known to most statisticians. It consists of a linear regression of one or more responses **Y** onto a number of principal component scores from a predictor matrix **X** (Næs and Martens, 1988).

PLSR is also a linear regression onto a number of components from **X**, but whereas principal component analysis maximizes the variance of the scores, PLS maximizes the covariance between the scores and the response. The idea is that this should give components that are more relevant for the response. Typically, PLSR achieves the same (or smaller) prediction error as PCR, with fewer components. A good introduction to PLSR and PCR can be found in Martens and Næs (1989). A review of PLSR is given in Wold et al. (2001) (in fact, all of that issue of Chemolab is dedicated to PLSR). Frank and Friedman (1993) provides a more technical treatment, from a statistical viewpoint.

PLSR and PCR are commonly used in situations where there are collinearities or near-collinearities in **X**, for instance when there are more variables than observations. This is a very common situation in fields like chemometrics, where various types of spectroscopic data are often used to predict other measurements.

There are other regression methods that can be applied to such data, for instance ridge regression. Studies have indicated that in terms of prediction error, ridge regression can perform slightly better than PLSR. However, one of the major advantages of PLSR and PCR is interpretation. In addition to a prediction equation, one gets score and loading vectors

A.5 Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity

Johannes Ranke*, Frauke Stock, Anja Müller, Stefan Stolte, Reinhold Störmann, Ulrike Bottin-Weber, and Bernd Jastorff

Ecotoxicology and Environmental Safety **67** (3) 430-438

Regarding the great structural variability of the currently expanding group of ionic liquids, it is highly desirable to understand the basic factors affecting their toxicity in different biological systems. The present study of a set of 74 ionic liquids with imidazolium, pyrrolidinium, pyridinium, quinolinium, quaternary phosphonium and quaternary ammonium cations and the comparatively small anions Cl^-, Br^-, BF_4^- or PF_6^- demonstrates the influence of the cation lipophilicity on the cytotoxicity in IPC-81 leukemia cells from rats. The scope of this correlation is limited to ionic liquids with these or similarly small anions that are sufficiently nonreactive under physiological and chromatographic conditions and whose cation lipophilicity does not exceed a certain threshold.

doi: 10.1016/j.ecoenv.2006.08.008

A.6 Effects of different head groups and modified side chains on the cytotoxicity of ionic liquids

Stefan Stolte*, Jürgen Arning, Ulrike Bottin-Weber, William-Robert Pitner, Urs Welz-Biermann, Bernd Jastorff, and Johannes Ranke

Green Chemistry **9** (8) 760-767

In this study, the influence of different head groups, functionalised side chains and anions of ionic liquids on the marine bacteria Vibrio fischeri, the limnic green algae Scenedesmus vacuolatus and the fresh water plant Lemna minor was investigated. The aim of these experiments is to improve the knowledge base for the molecular design of ionic liquids leading to a reduced (eco)toxicological hazard potential. The analysed set of about 40 ionic liquids confirmed the interdependency between lipophilicity — as derived from gradient HPLC — and (eco)toxicity. The toxicity was clearly reduced for the test organisms (partially by six to seven orders of magnitude) when short functionalised side chains were used instead of non-polar alkyl chains. Furthermore, we could demonstrate strong interactions of hydrophobic ionic liquid cations with two different types of common biological lipid bilayers, indicating that the membrane system of organisms is probably a primary target site of toxic action. These systematic studies are addressed to producers, developers and downstream users of ionic liquids in different fields of application, to facilitate the selection of (eco)toxicologically favourable structural elements and thus to contribute to the design of inherently safer ionic liquids.

doi: 10.1039/b711119c

A.7 Design of sustainable chemical products — the example of ionic liquids

Johannes Ranke*, Stefan Stolte, Reinhold Störmann, Jürgen Arning, and Bernd Jastorff

Chemical Reviews **107** (6) 2183-2206

doi: 10.1021/cr050942s

A.8 Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids

Stefan Stolte*, Marianne Matzke, Jürgen Arning, Andrea Böschen, William-Robert Pitner, Urs Welz-Biermann, Bernd Jastorff, and Johannes Ranke

Green Chemistry **9** (11) 1170-1179

In this study, the influence of different head groups, functionalised side chains and anions of ionic liquids on the marine bacteria Vibrio fischeri, the limnic green algae Scenedesmus vacuolatus and the fresh water plant Lemna minor was investigated. The aim of these experiments is to improve the knowledge base for the molecular design of ionic liquids leading to a reduced (eco)toxicological hazard potential. The analysed set of about 40 ionic liquids confirmed the interdependency between lipophilicity — as derived from gradient HPLC — and (eco)toxicity. The toxicity was clearly reduced for the test organisms (partially by six to seven orders of magnitude) when short functionalised side chains were used instead of non-polar alkyl chains. Furthermore, we could demonstrate strong interactions of hydrophobic ionic liquid cations with two different types of common biological lipid bilayers, indicating that the membrane system of organisms is probably a primary target site of toxic action. These systematic studies are addressed to producers, developers and downstream users of ionic liquids in different fields of application, to facilitate the selection of (eco)toxicologically favourable structural elements and thus to contribute to the design of inherently safer ionic liquids.

doi: 10.1039/b711119c

A.9 Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups, functionalised side chains and anions of ionic liquids on acetylcholinesterase

Jürgen Arning*, Stefan Stolte, Andrea Böschen, Frauke Stock, William-Robert Pitner, Urs Welz-Biermann, Bernd Jastorff, and Johannes Ranke

Green Chemistry **10** (1) 47-58

To contribute to a deeper insight into the hazard potential of ionic liquids to humans and the environment, an acetylcholinesterase (AchE) inhibition screening assay was used to identify toxicophore substructures and interaction potentials mediating enzyme inhibition. The positively charged nitrogen atom, a widely delocalised aromatic system, and the lipophilicity of the side chains connected to the cationic head groups can be identified as the key structural elements in binding to the enzymes active site. With respect to this, the dimethylaminopyridinium, the quinolinium and the pyridinium head groups exhibit a very strong inhibitory potential to the enzyme with IC50 values around 10 μM. In contrast, the polar and non-aromatic morpholinium head group is found to be only weakly inhibiting to the enzyme activity, with IC50 values > 500 μM. The introduction of polar hydroxy, ether or nitrile functions into the alkyl side chain is shown to be a potent structural alteration to shift the corresponding ionic liquids to a lower inhibitory potential. Supporting this fact, for a series of imidazolium cations, a QSAR correlation was set up by the linear regression of the log IC50 versus the logarithm of the HPLC-derived lipophilicity parameter k0. Additionally, a broad set of anion species (inorganic, organic and complex borate anions), commonly used as ionic liquid counterions, was tested and the vast majority exhibited no effect on AchE. Only the fluoride and fluoride containing anion species which readily undergo hydrolytic cleavage can be identified to act as AchE inhibitors.

doi: 10.1039/b712109a

A.10 Risk assessment of biocides in roof paint I: Experimental determination and modelling of biocide leaching from roof paint

Christian Jungnickel, Frauke Stock, Thomas Brandsch, and Johannes Ranke*

Environmental Science and Pollution Research **15** (3) 268-285

Background, Aim and Scope Many surface coatings, including roof paints, contain biocides. It is generally not known to what extent roof paint biocides leach from the paint, and consequently, what concentration the biocide may attain in a rainwater collection system. To this end the leaching of specific biocides from a variety of German roof paints was investigated and the resulting concentrations in collected rain water were estimated.
Materials and Methods A laboratory simulation was used to determine the time dependant leaching rate of the biocide from the paint into synthetic rainwater. The concentrations of biocide in the leachate were quantified using HPLC. The course of the leachate concentrations over time was fitted using a simple mathematical model. This was then used to estimate concentrations of biocides in a typical household rainwater collection system over time.
Results Surprisingly, the biocides found in the paints did not always concur with the declared biocides. Concerning the modelling of runoff concentrations, it was found that — under the model assumptions — the rain intensity and cumulative raining time after application are the dominant factors influencing the concentration of the biocide. At the highest modelled rain intensity of 40 mm/hour it only takes about 2 hours to reach peak concentrations lower than 0.1 mg/L, at 0.3 mm/hour it takes about 10 hours to reach peak concentrations of 1.3, 0.9, 5.2 and 1.1 mg/L for terbutryn from Emalux paint, terbutryn from Südwest paint, carbendazim from Emalux paint, and carbendazim from MIPA paint, respectively.
Discussion The results confirm that biocides leached from roof paint will be present in roof runoff. The highest estimated peak concentrations are close to the water solubility of the respective biocides. This indicates that the model assumption of a concentration independent leaching rate will tendentially lead to an overestimation of the leached concentrations under these circumstances. However, under most circumstances such as higher rain intensities, and longer time after peak concentrations have been reached, the runoff concentrations are far from the solubility limit, and therefore it is proposed that the model assumptions are tenable.

Conclusions The leaching of biocides from roof paints can be roughly assessed using a relatively simple approach. The declaration of biocidal ingredients in roof paints should be improved and information on their biocide leaching behaviour should be made available. Furthermore, the estimations should be evaluated by a field study.
Recommendations and Perspectives The leaching study indicated that the concentrations of selected biocides can reach significant levels, especially after low intensity rainfall. Taking into account the inherent biological activity of the substances under scrutiny, it can already be concluded that it is not advisable to use runoff water from roofs freshly painted with biocide containing roof paints. These results have been complemented by a literature search of biological effects of the investigated biocides, ecotoxicological tests with several species and a risk analysis for organisms exposed to runoff water. This will be presented in Part 2 of this contribution.

doi: 10.1065/espr2007.12.465

A.11 Structure-activity relationships for the impact of selected isothiazol-3-one biocides on glutathione metabolism and glutathione reductase of the human liver cell line Hep G2

Jürgen Arning*, Ralf Dringen, Maike Schmidt, Anette Thiessen, Stefan Stolte, Marianne Matzke, Ulrike Bottin-Weber, Birgit Caesar-Geertz, Bernd Jastorff, and Johannes Ranke

Toxicology **246** (2-3) 203-212

To investigate the toxic mode of action of isothiazol-3-one biocides the four compounds N-methylisothiazol-3-one (MIT), 5-chloro-N-methylisothiazol-3-one (CIT), N-octylisothiazol-3-one (OIT) and 4,5-dichloro-N-octylisothiazol-3-one (DCOIT) were purified and tested as single chemical entities for their effects on the human hepatoblastoma cell line Hep G2 and on isolated and cellular glutathione reductase GR). The two chlorinated substances CIT and DCOIT significantly decreased the amount of total cellular glutathione (GSx) in a dose and time dependent manner. Concomitantly, an increase in the level of oxidised glutathione (GSSG) was observed. The resulting shift in the GSH/GSSG ratio entailing the breakdown of the cellular thiol reduction potential was accompanied by necrotic morphological changes like swelling of the plasma membrane and subsequent lysis of the cells. Additionally, CIT and DCOIT were found to inhibit cellular GR in the cells in a concentration dependent manner. The T-SAR-based (thinking in terms of structure-activity relationships) comparison of the chlorine-substituted structures CIT and DCOIT with their non-chlorinated and less active analogues MIT and OIT identified the chlorine substituents and the resulting reaction mechanisms to be the key structural mediators of the observed toxic effects. Furthermore, differences in the activity of both chlorinated substances could be explained using the T-SAR approach to link the lipophilicity and the intrinsic glutathione-reactivity of the compounds to the expected target site concentrations inside the cells.

doi: 10.1016/j.tox.2008.01.011

A.12 Developing and Disseminating NOP: An Online, Open-Access, Organic Chemistry Teaching Resource To Integrate Sustainability Concepts in the Laboratory

Johannes Ranke*, Müfit Bahadir, Marco Eissen, and Burkhard König

Journal of Chemical Education **85** (7) 1000-1005

To foster greater awareness of sustainability issues in chemistry among future professional chemists, traditional course content must be revised. We have collected and developed material that allows students and teachers of organic chemistry to assess reactions beyond experimental set ups, reaction mechanisms, and chemical yields. The NOP resource we use and distribute freely online includes additional parameters that should be considered in the organic chemistry laboratory: atom economy of chemical transformations; energy efficiency of chemical transformations; questions of waste; renewable feedstocks; toxicity and ecotoxicity; and safety measures for the chemicals used. We report on the development, use, and international adoption of NOP project efforts to promote sustainable practices in organic chemistry laboratories.

doi: 10.1021/ed085p1000

A.13 Analysing cytotoxic effects of selected isothiazol-3-one biocides using the Toxic Ratio concept and the Structure-Activity considerations

Jürgen Arning*, Marianne Matzke, Stefan Stolte, Frauke Nehen, Ulrike Bottin-Weber, Andrea Böschen, Salha Abdulkarim, Bernd Jastorff, and Johannes Ranke

Chemical Research in Toxicology **22** (12) 1954-1961

To demonstrate how baseline toxicity can be separated from other more specific modes of toxic action and to address possible pitfals when dealing with hydrophobic substances, the four isothiazol-3-one biocides N-methylisothiazol-3-one (MIT), 5-chloro-N-methylisothiazol-3-one (CIT), N-octylisothiazol-3-one (OIT), and 4,5-dichloro-N-octylisothiazol-3-one (DCOIT) as an example for reactive electrophilic xenobiotics were tested for their cytotoxic effects on the human hepatoblastoma cell line Hep G2, on the marine bacterium Vibrio fischeri, and on the limnic green alga Scenedesmus vacuolatus. In each of the three test systems, toxic effects were observed in a consistent pattern. The two chlorinated compounds and OIT were found to be significantly more toxic than MIT. As compared to baseline toxicants, the small and polar MIT and CIT exhibited pronounced excess toxicity in each of the three test systems that is presumably triggered by their intrinsic reactivity toward cellular thiols. In contrast, OIT and DCOIT showed mainly toxicities that could be explained by their hydrophobicity. Analyzing and comparing these results using the toxic ratio concept and with data that indicate a dramatic depletion of cellular glutathione levels after incubation with DCOIT reveals that for highly hydrophobic substances, baseline level toxicity in an assay for acute toxicity can lead to an oversight of other more specific modes of toxic action that may cause significant effects that might be less reversible than those caused by unreactive baseline toxicants. This possibility should be taken into account in the hazard assessment of chemicals that are both hydrophobic and reactive.

doi: 10.1021/tx900263m

A.14 Explaining ionic liquid water solubility in terms of cation and anion hydrophobicity

Johannes Ranke*, Alaa Othman, Ping Fan, and Anja B. Müller

International Journal of Molecular Science **10** (3) 1271-1289

Full paper reprinted here

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Article

Explaining Ionic Liquid Water Solubility in Terms of Cation and Anion Hydrophobicity

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Abstract: The water solubility of salts is ordinarily dictated by lattice energy and ion solvation. However, in the case of low melting salts also known as ionic liquids, lattice energy is immaterial and differences in hydrophobicity largely account for differences in their water solubility. In this contribution, the activity coefficients of ionic liquids in water are split into cation and anion contributions by regression against cation hydrophobicity parameters that are experimentally determined by reversed phase liquid chromatography. In this way, anion hydrophobicity parameters are derived, as well as an equation to estimate water solubilities for cation-anion combinations for which the water solubility has not been measured. Thus, a new pathway to the quantification of aqueous ion solvation is shown, making use of the relative weakness of interactions between ionic liquid ions as compared to their hydrophobicities.

Keywords: Ionic liquids; Water solubility; Cations; Anions; Lipophilicity.

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1. Introduction

The presumed discovery of the first ionic liquid by Walden [1] took place in 1914. After that, it took over 80 years for the bloom of ionic liquid research to appear, starting with the discovery of a new class of air and water stable ionic liquids by Wilkes in 1992 [2]. It will maybe take another 80 years to realize the full potential of these substances, or, as Uwe Vagt (BASF) put it at the Intertech Pira Conference on Ionic Liquids in Prague in October 2007, "We are still at the beginning." Numerous contributions [3, 4, 5, 6, 7, 8] have increased our understanding of basic properties, and to predict physical [9, 10, 11, 12] or toxicological [13, 14, 15] properties of ionic liquids. Especially in the context of green and sustainable chemistry, there is a vision of a molecular design of ionic liquids which would combine technical advantages with a set of favorable properties like ready availability and low risk for health and environment [16, 17].

The quality of our recently found correlation between cation hydrophobicity and cytotoxicity for ionic liquids with relatively small anions [14] inspired us to also investigate the correlation of cation hydrophobicity with water solubility data at 293 K that had previously been generated in our labs. Because of the good correlation found within this dataset (see below), we complemented our water solubility data with data from the scientific literature.

The model we use for the water solubility of ionic liquids is:

$$\log x_{\mathrm{IL,w}}^{\mathrm{sat}} = -m \log k_{0,\mathrm{c}} + c_{\mathrm{a}} + \epsilon, \tag{1}$$

where $x_{\mathrm{IL,w}}^{\mathrm{sat}}$ is the mole fraction solubility of the ionic liquid at 293 K, $k_{0,\mathrm{c}}$ is the cation hydrophobicity parameter as defined previously [14], c_{a} is an anion specific constant expressing their hydrophobicity, and ϵ is the random variable describing deviations from the model with unknown or unspecified causes. The slope m should ideally be unity if all model assumptions are satisfied.

Briefly, the model is based on the idea that the excess molar free energy of dissolving the ionic liquid in water $\Delta_{\mathrm{IL,w}} g^{\mathrm{E}} = RT \ln \gamma_{\mathrm{IL,w}}$ can be expressed as the sum of a cation and an anion contribution, where the contribution of the cation is given by $\log k_{0,\mathrm{c}}$ plus an unknown constant, and the contribution of the anion is extracted by a least squares fit of the model to the data.

2. Theory

For the theoretical discussion of the model we need a notation that covers both a) cation partitioning in reversed phase HPLC, quantified by the capacity factor k_{c} extrapolated to the conditions at the beginning of an acetonitrile gradient and b) the ionic liquid - water equilibrium at saturation, specified by the equilibrium mole fraction of ionic liquid in the water phase $x_{\mathrm{w}}^{\mathrm{sat}}$.

We first denote the chemical potential of an ionic liquid in solvent s as:

$$\mu_{\mathrm{IL,s}} = \mu_{\mathrm{IL}}^{\bullet} + RT \ln \gamma_{\mathrm{IL,s}}\, x_{\mathrm{IL,s}} \tag{2}$$

using the pure ionic liquid as the standard state. Restricting our model to ionic liquids that are composed of one cation type and one anion type at a 1:1 ratio (covering the majority of what is called ionic liquids today), we split $\mu_{\mathrm{IL,s}}$ into the sum of the chemical potentials of cation and anion:

$$\mu_{\mathrm{IL,s}} = \mu_{\mathrm{c,s}} + \mu_{\mathrm{a,s}} \tag{3}$$

with

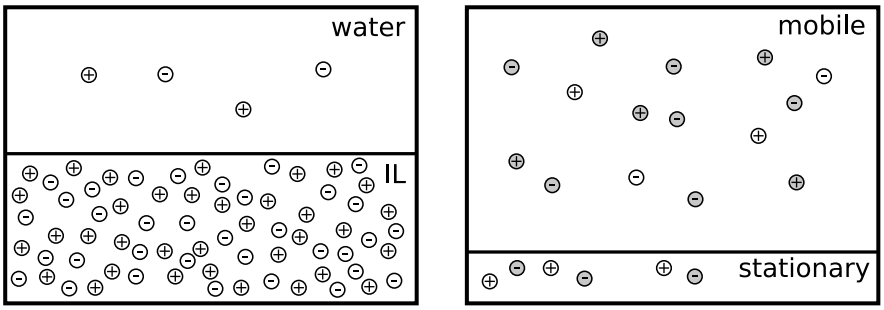
$$\mu_{\mathrm{c,s}} = \mu_{\mathrm{c}}^{\bullet} + RT \ln \gamma_{\mathrm{c,s}}\, x_{\mathrm{c,s}} \tag{4}$$

$$\mu_{\mathrm{a,s}} = \mu_{\mathrm{a}}^{\bullet} + RT \ln \gamma_{\mathrm{a,s}}\, x_{\mathrm{a,s}} \tag{5}$$

using the pure ionic liquid as the standard state as in equation 2. Note that these ion chemical potentials are not defined *sensu strictu*, because ions of one polarity can not be solvated without the presence of suitable counteranions. However, for our discussion it is convenient to use the terminology of chemical potentials and activity coefficients of ions, although extrathermodynamic assumptions are necessary to determine them [18].

We will now use this notation in order to discuss the two scenarios mentioned above, which are depicted in Figure 1:

Figure 1. Equilibria of ionic liquid and water (left) and of ionic liquid cations between stationary and mobile phase in buffered reversed phase HPLC (right). Open circles are ionic liquid cations and anions, grey circles are buffer ions present in the HPLC mobile phase. In the case of the water saturation, electroneutrality in the water can only be kept if the concentrations of IL cations and anions are equal. In the case of buffered HPLC, IL cations and anions can partition largely independent of each other if sufficient concentrations of counterions are present in both phases.



In the case of the saturation of water with an ionic liquid, electroneutrality dictates that $x_{\mathrm{c,w}} = x_{\mathrm{a,w}} = x_{\mathrm{IL,w}}$ in the aqueous phase w. Neglecting the amount of water partitioning into the IL ($x^{\mathrm{sat}}_{\mathrm{IL,IL}} \approx 1$, Assumption 1), and assuming that it does not significantly influence the chemical environment of the IL ($\gamma^{\mathrm{sat}}_{\mathrm{IL,IL}} \approx 1$, Assumption 2), the chemical potential of the IL at water saturation is approximately equal to the standard chemical potential of the standard state, i.e. $\mu^{\mathrm{sat}}_{\mathrm{IL,IL}} \approx \mu^{\bullet}_{\mathrm{IL}}$.

At the point of the saturation equilibrium, the chemical potentials of the IL have to be equal, i.e.

$$\mu^{\mathrm{sat}}_{\mathrm{IL,IL}} = \mu^{\mathrm{sat}}_{\mathrm{IL,w}} \tag{6}$$

Using the standard state as an approximation for the left hand side of equation 6, and equation 2 for its right hand side,

$$\mu^{\bullet}_{\mathrm{IL}} = \mu^{\bullet}_{\mathrm{IL}} + RT \ln \gamma_{\mathrm{IL,s}}\, x_{\mathrm{IL,s}} \tag{7}$$

we can rearrange the result to read

$$RT \ln x^{\mathrm{sat}}_{\mathrm{IL,w}} = -RT \ln \gamma_{\mathrm{IL,w}} \tag{8}$$

We now assume that the excess molar free energy of dissolving the IL in water $\Delta_{\mathrm{IL,w}} g^{\mathrm{E}} = RT \ln \gamma_{\mathrm{IL,w}}$ can be expressed as the sum of a cation and an anion contribution (Assumption 3), i.e.

$$RT \ln x^{\mathrm{sat}}_{\mathrm{IL,w}} = -RT \ln \gamma_{\mathrm{c,w}} - RT \ln \gamma_{\mathrm{a,w}} \tag{9}$$

or, if we divide by RT and move to base ten for the logarithmic expressions

$$\log x^{\mathrm{sat}}_{\mathrm{IL,w}} = -\log \gamma_{\mathrm{c,w}} - \log \gamma_{\mathrm{a,w}} \tag{10}$$

which gives us a relation between cation and ion hydrophobicities, expressed by their activity coefficients in water, and water solubility.

If we look at the cation partitioning between mobile and stationary phases in reversed phase HPLC, we can assume that electroneutrality in both phases is guaranteed by a sufficient concentration of counteranions present in them (Assumption 4). This only appears reasonable if the buffer anion is sufficiently hydrophobic to be able to enter the stationary interphase at the chemically modified silica surface, or if there is a sufficient surface concentration of negatively charged sites with loosely bound counterions, for example dissociated silanol groups.

If this condition is satisfied, and if we neglect ion pairing between ionic liquid cations and anions (Assumption 5), we can denote the cation partitioning coefficient between stationary and mobile phase

$$K_{\mathrm{c,stm}} = \frac{x_{\mathrm{c,st}}}{x_{\mathrm{c,m}}} = \frac{\gamma_{\mathrm{c,m}}}{\gamma_{\mathrm{c,st}}}, \tag{11}$$

neglecting any electrostatic contribution to the partitioning. In logarithmic form, this can be written as

$$\log K_{\mathrm{c,stm}} = \log \gamma_{\mathrm{c,m}} + c_1 \tag{12}$$

where the c_1 represents the contribution of the activity coefficient of the cation in the stationary phase, but can potentially also represent an electrostatic contribution, which would be independent of the type of the cation. If we assume that the activity coefficient of the cation in the stationary phase is approximately invariant for the range of cations studied (Assumption 6), c_1 can be taken as a constant.

In a previous study, we have shown how a measure for such a partition coefficient $K_{\mathrm{c,stm}}$ can be extracted from retention times in gradient HPLC. More specifically, we use the capacity factor $\log k_{0,\mathrm{c}}$

for the situation at the beginning of the gradient, i.e. for the situation where the concentration of organic modifier is zero, and the mobile phase only consists of an ammonium acetate buffer, as a measure for the cation partitioning coefficient $K_{\mathrm{c,stm_0}}$, also at the beginning of the HPLC gradient [14].

In partition chromatography, the constant of proportionality c_2 in the equation for the capacity factor k

$$k = c_2 K_{\mathrm{stm}} \tag{13}$$

is just the phase ratio of stationary and mobile phase in the separation column. Since the volumes of stationary and mobile phase in reversed phase HPLC are ill-defined, we treat c_2 as a constant without explicit physical interpretation.

Combining equations 12 and 13, we find that the log of the capacity factor $\log k_{0,\mathrm{c}}$ is just the cation activity coefficient in the mobile phase m_0 at the beginning of the gradient plus a constant

$$\log k_{0,\mathrm{c}} = \log \gamma_{\mathrm{c,m_0}} + c_3. \tag{14}$$

The constant c_3 incorporates chemical interactions of the cations with the stationary phase, possibly an electrostatic contribution, and the correlate to the phase ratio c_2 from equation 13.

Under the final assumption that the activity coefficient of the cation in pure water is approximately equal to its activity coefficient in the mobile phase at the beginning of the gradient, in our case an ammonium acetate buffer, (Assumption 7), we can combine equations 10 and 14 to

$$\log x^{\mathrm{sat}}_{\mathrm{IL,w}} = -\log k_{0,\mathrm{c}} - \log \gamma_{\mathrm{a,w}} + c_3 \tag{15}$$

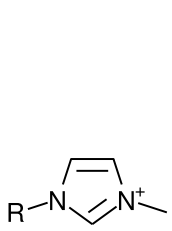
Therefore the slope m in the model equation 1 for the water solubility of ionic liquids repeated below is ideally equal to unity. The anion constant c_{a} equals $c_3 - \log \gamma_{a,w}$.

$$\log x^{\mathrm{sat}}_{\mathrm{IL,w}} = -m \log k_{0,\mathrm{c}} + c_{\mathrm{a}} + \epsilon \tag{16}$$

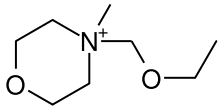
3. Materials and Methods

Ionic liquid nomenclature. In this study, the acronym scheme established at the UFT Center for Environmental Research and Sustainable Technology, University of Bremen is used for cations as illustrated in Table 1. Generally, numbers refer to alkyl chains (as in IM14 where the numbers refer to one methyl and one alkyl substituent on the nitrogens of the imidazolium cation) similar to their use in Wiswesser line notation. However, sometimes they are also pragmatically used to designate the site of substitution, as in Py4-3Me, where "-3Me" indicates 3-methyl substitution of the N-butylpyridinium cation.

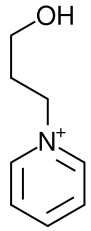
Table 1. Chemical structures of the cations treated in this study, along with their acronyms. In the acronyms, IM, Mor, N, Pip, Pyr and Py are used for the imidazolium, morpholinium, ammonium, piperidinium, pyrrolidinium and pyridinium head groups. n designates linear alkyl chains with varying length, where n is the number of carbon atoms in the chain. In the graphs, these chains correspond to the residues R.



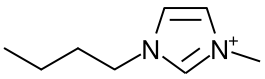
IM1n



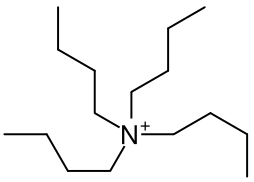
Mor11O2



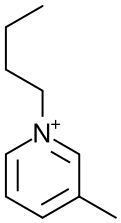
Py3OH



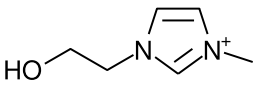
IM14



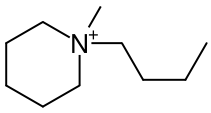
N4444



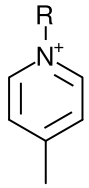
Py4-3Me



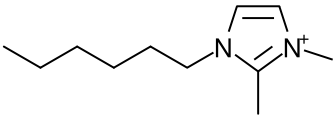
IM12OH



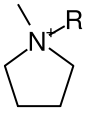
Pip14



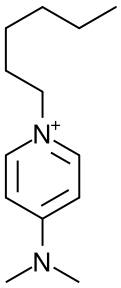
Pyn-4Me



IM16-2Me



Pyr1n



Py6-4NMe2

Ionic liquids listed in Table 2 were used as received from Merck KGaA, Darmstadt, Germany. The first three entries in the table were used for the determination of their cation hydrophobicities as specified below. The remaining ionic liquids were used in the determination of their water solubility.

Table 2. Ionic liquids used for generation of original data for this study.

Ionic liquid	Acronym
1-ethyl-3-methylimidazolium chloride	IM12 Cl
1-(3-hydroxypropyl)pyridinium chloride	Py3OH Cl
4-(ethoxymethyl)-4-methylmorpholinium chloride	Mor11O2 Cl
1-butyl-3-methylimidazolium hexafluorophosphate	IM14 PF6
1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	IM14 (CF3SO2)2N
1-hexyl-3-methylimidazolium hexafluorophosphate	IM16 PF6
1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	IM16 (CF3SO2)2N
1-hexyl-3-methylimidazolium tris(trifluoromethylsulfonyl)methide	IM16 (CF3SO2)3C
1-hexyl-3-methylimidazolium trifluorotris(pentafluoroethyl)phosphate	IM16 (C2F5)3PF3
1-octyl-3-methylimidazolium hexafluorophosphate	IM18 PF6
1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide	IM18 (CF3SO2)2N
1-(3-hydroxypropyl)pyridinium bis(trifluoromethylsulfonyl)imide	Py3OH (CF3SO2)2N
4-(dimethylamino)-1-hexylpyridinium bis(trifluoromethylsulfonyl)imide	Py6-4NMe2 (CF3SO2)2N
1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide	Pyr14 (CF3SO2)2N
1-butyl-1-methylpyrrolidinium trifluorotris(pentafluoroethyl)phosphate	Pyr16 (C2F5)3PF3
1-hexyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide	Pyr16 (CF3SO2)2N

Cation hydrophobicity parameters $\log k_{c,0}$ were taken from [14] and [19]. The $\log k_{c,0}$ parameters for the cations 1-ethyl-3-methylimidazolium, 1-(3-hydroxypropyl)pyridinium, and 4-(ethoxymethyl)-4-methylmorpholinium newly presented here were derived from capacity factors calculated from isocratic retention times on the same reversed phase HPLC column type (Polaris Ether C18 from Varian, 150 mm, 3 mm inner diameter, 5 m particle size). Specifically, for each mobile phase composition, i.e. 2.5, 5, 10, 15 and 20 % gradient grade acetonitrile complemented with 0.25 % acetic acid (p.a., both Fluka, Buchs, Switzerland), a linear correlation was established between capacity factors under these conditions and previously established [14, 19] $\log k_{c,0}$ values. For the three cations without such previously established values, the mean of the predictions based on these linear correlations was used as $\log k_{c,0}$ in Table 3.

Determination of water solubility was carried out based on OECD guideline 105 for testing of chemicals. After establishment of approximate water solubility by a preliminary visual test, six aliquots of 100 mL water were equilibrated with the amount of ionic liquid resulting in five-fold oversaturation according to the preliminary result in a water bath at 30 degrees C, and repeatedly mixed by thoroughly shaking them manually. After 24, 48, and 72 hours two aliquots were transferred to a water bath at 20 degrees C. Each of those aliquots was gently centrifuged after a second 24 h equilibration period in the second water bath, and the ionic liquid concentration in the water phase was determined by reversed phase HPLC. The experimental uncertainty of the water solubility determinations depends on the soluble concentration and is better for ionic liquids exhibiting higher water solubility. Generally, deviations should be lower than 0.1 log units.

HPLC determination of ionic liquid concentrations in the saturated solutions was carried out using isocratic reversed phase HPLC on a Hewlett Packard 1100 Series system, equipped with online degasser, variable wavelength UV detector and a Bruker esquire ESI-MS ion trap detector. For the UV-absorbing cations, a Polaris Ether column from Varian also used for the hydrophobicity determination was used, with 20 mM KH_2PO_4 and 3.9 mM H_3PO_4 on channel A and gradient grade acetonitrile on channel B, using a suitable percentage of acetonitrile for each cation, and UV detection at 212 nm. For the cations without UV absorption, the same acetic acid/acetonitrile eluent system used in the hydrophobicity determination was used isocratically on the Polaris Ether RP 18 column, in order to have better compatibility with the ESI interface, again adapting the acetonitrile percentage to the analyte cation. For the quantification by ESI-MS, only a concentration range of two decades at maximum could be used, because of the nonlinear relation between concentration and peak area. In both cases, the pH of the eluent has to be around 3, in order to avoid tailing and overly long retention times. Conversely, for the most hydrophilic cations, a MonoChrom MS column from Varian was used for quantification, in order to obtain sufficient retention.

Ionic liquid concentrations at saturation derived from cation analysis via HPLC-ESI-MS were double checked by determining peak areas of the hydrophobic anions bis(trifluoromethlysulfonyl)imide and trifluorotris(pentafluoroethyl)phosphate in the negative mode of the ion trap detector on the same column, but with only 0.1 % to 0.25 % acetic acid as the aqueous part and 60 to 70 % MeOH. They diverged by less than ten percent, and the mean of cation and anion concentrations was taken as the final result.

4. Results

The data analysed using the model derived above are listed in Table 3. Most of the cation hydrophobicity data has already been published elsewhere by our group [14, 19]. The water solubility data generated by us has not been published previously.

Table 3. Water solubility at temperatures within 293.15 ± 5 K and cation hydrophobicity. Data without source attribution are published here for the first time. Data with a mole fraction solubility greater than 0.05 are not considered, because this would conflict with Assumptions 1 and 2 stated in the text. Data from Branco et al. [20] were not considered as they strongly diverge from other sources for an unknown reason.

	Cation hydrophobicity			IL water solubility		
Cation[a]	$\log k_{0,c}$		Anion[b]	$\log_{10} x^{sat}_{IL,w}$	T [K]	
IM16	1.2	[14]	$(C_2F_5)_3PF_3$	-5.93	293.15	
Pyr14	0.57	[14]	$(C_2F_5)_3PF_3$	-5.43	293.15	
Py8-4Me	2	[14]	$(C_2F_5SO_2)_2N$	-5.4	298.15	[21]
Py8-4Me	2	[14]	$(CF_3SO_2)_2N$	-5.09	298.15	[21]
IM16	1.2	[14]	$(CF_3SO_2)_3C$	-5.04	293.15	
Py8-4Me	2	[14]	AsF_6	-4.91	298.15	[21]
Pyr18	1.9	[14]	$(CF_3SO_2)_2N$	-4.71	298	[22]

Table 3. Cont.

Cation[a]	Cation hydrophobicity		Anion[b]	IL water solubility		
	$\log k_{0,c}$			$\log_{10} x_w^{sat}$	T [K]	
IM18	1.9	[14]	$(C_2F_5SO_2)_2N$	-4.7	298	[23]
Py8-4Me	2	[14]	$(C_4F_9)SO_3$	-4.63	298.15	[21]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.6	298	[23]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.59	293.15	
Py6-4NMe2	1.8	[19]	$(CF_3SO_2)_2N$	-4.53	293.15	
Py6-4NMe2	1.8	[19]	$(CF_3SO_2)_2N$	-4.53	296.5	[24]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.5	288.15	[25]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.49	293.15	[25]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.47	298.15	[25]
IM14	0.67	[14]	$(CF_3SO_2)_3C$	-4.44	296.5	[24]
IM17	1.6	[14]	$(CF_3SO_2)_2N$	-4.31	288.15	[25]
IM17	1.6	[14]	$(CF_3SO_2)_2N$	-4.3	293.15	[25]
IM17	1.6	[14]	$(CF_3SO_2)_2N$	-4.29	298.15	[25]
IM18	1.9	[14]	$(C_4F_9)SO_3$	-4.23	298	[26]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-4.18	293.15	
IM16-2Me	1.4	[14]	$(CF_3SO_2)_2N$	-4.15	296.5	[24]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.14	296.5	[24]
Pyr16	1.2	[14]	$(CF_3SO_2)_2N$	-4.12	293.15	
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.1	298	[26]
IM18	1.9	[14]	$(CF_3SO_2)_2N$	-4.1	293.15	[27]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-4.05	288.15	[25]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-4.05	293.15	[25]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-4.03	296.5	[24]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-4.02	298.15	[25]
IM18	1.9	[14]	PF_6	-3.95	288.15	[28]
IM18	1.9	[14]	PF_6	-3.93	293.15	
IM18	1.9	[14]	PF_6	-3.92	293.15	[28]
IM18	1.9	[14]	PF_6	-3.9	298.15	[28]
IM16	1.2	[14]	$(CF_3SO_2)_2N$	-3.86	293.15	[27]
Pip14	0.68	[19]	$(CF_3SO_2)_2N$	-3.78	298	[22]
IM15	0.92	[14]	$(CF_3SO_2)_2N$	-3.74	288.15	[25]
IM15	0.92	[14]	$(CF_3SO_2)_2N$	-3.73	293.15	[25]
IM15	0.92	[14]	$(CF_3SO_2)_2N$	-3.71	298.15	[25]
Py4-3Me	0.73	[14]	$(CF_3SO_2)_2N$	-3.7	296.5	[24]
Py4-4Me	0.73	[14]	$(CF_3SO_2)_2N$	-3.69	298	[26]
Py8-4Me	2	[14]	$PhBF_3$	-3.6	298.15	[21]

Table 3. Cont.

Cation[a]	Cation hydrophobicity		Anion[b]	IL water solubility		
	$\log k_{0,c}$			$\log_{10} x_w^{sat}$	T [K]	
Pyr14	0.57	[14]	$(CF_3SO_2)_2N$	-3.59	298	[22]
Pyr14	0.57	[14]	$(CF_3SO_2)_2N$	-3.57	293.15	
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.54	288.15	[25]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.53	293.15	[25]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.51	298.15	[25]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.51	298	[26]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.5	296.5	[24]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.5	293.15	
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.49	294.15	[29]
IM14	0.67	[14]	$(CF_3SO_2)_2N$	-3.46	293.15	[27]
IM18	1.9	[14]	PF_6	-3.46	295	[30]
IM16	1.2	[14]	PF_6	-3.45	288.15	[28]
IM16	1.2	[14]	PF_6	-3.41	293.15	[28]
IM16	1.2	[14]	PF_6	-3.36	298.15	[28]
IM16	1.2	[14]	PF_6	-3.35	293.15	
IM13	0.42	[14]	$(CF_3SO_2)_2N$	-3.29	288.15	[25]
IM13	0.42	[14]	$(CF_3SO_2)_2N$	-3.28	293.15	[25]
IM13	0.42	[14]	$(CF_3SO_2)_2N$	-3.27	298.15	[25]
Mor11O2	0.17		$(CF_3SO_2)_2N$	-3.19	293.15	
IM14	0.67	[14]	$(C_4F_9)SO_3$	-3.15	298	[26]
IM12	0.22		$(CF_3SO_2)_2N$	-3.12	288.15	[25]
IM12	0.22		$(CF_3SO_2)_2N$	-3.1	296.5	[24]
IM12	0.22		$(CF_3SO_2)_2N$	-3.1	293.15	[25]
Py8-4Me	2	[14]	CF_3SO_3	-3.09	298.15	[21]
IM12	0.22		$(CF_3SO_2)_2N$	-3.08	293.15	[27]
IM12	0.22		$(CF_3SO_2)_2N$	-3.08	298.15	[25]
Py4-4Me	0.73	[14]	$(C_4F_9)SO_3$	-3.03	298	[26]
IM14	0.67	[14]	PF_6	-3	288.15	[28]
Py8-4Me	2	[14]	BF_4	-2.98	298.15	[21]
IM14	0.67	[14]	PF_6	-2.96	293.15	[28]
IM14	0.67	[14]	PF_6	-2.93	294	[31]
IM18	1.9	[14]	BF_4	-2.93	295	[30]
IM14	0.67	[14]	PF_6	-2.92	298.15	[28]
IM14	0.67	[14]	PF_6	-2.9	293.15	
IM14	0.67	[14]	PF_6	-2.9	296.5	[24]
IM14	0.67	[14]	PF_6	-2.89	295	[30]

Table 3. Cont.

Cation[a]	Cation hydrophobicity		Anion[b]	IL water solubility		
	$\log k_{0,c}$			$\log_{10} x_w^{sat}$	T [K]	
IM14	0.67	[14]	PF_6	-2.87	294.15	[29]
IM14	0.67	[14]	PF_6	-2.8	293.15	[27]
IM12	0.22		$B(CN)_4$	-2.46	296.5	[24]
Py3OH	-0.09		$(CF_3SO_2)_2N$	-2.43	293.15	
IM12OH	-0.28	[19]	$(CF_3SO_2)_2N$	-2.34	296.5	[24]
N4444	2.3	[14]	(6-2Et)2SS	-1.52	298	[32]

[a] Cation acronyms explained in Table 1

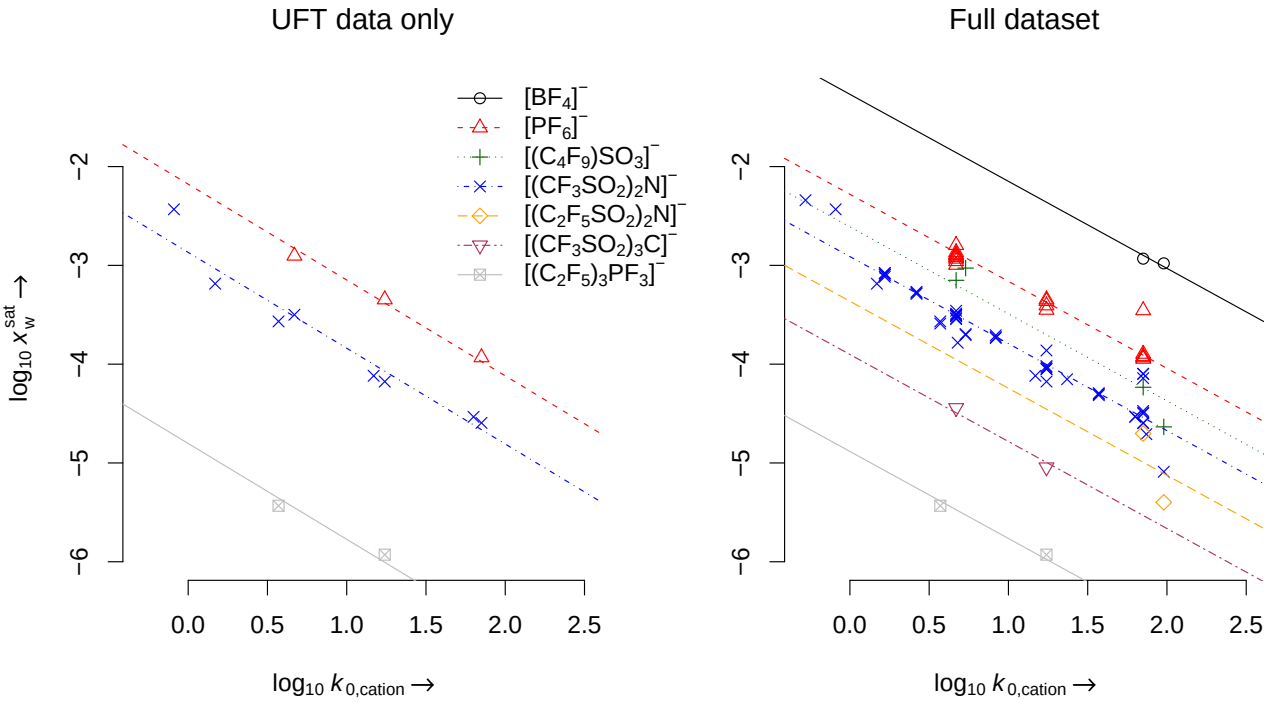
[b] (6-2Et)-2SS = bis(2-ethylhexyl)sulfosuccinate

In Figure 2, water solubilities of ionic liquids that form a second phase with water at room temperature are plotted against the hydrophobicity of their cation. The trend lines in the graph resulting from fitting the linear model specified in equation 1. Only data for anions combined with at least two different cations are shown.

The two available water solubility data for the $(C_2F_5SO_2)_2N$ anion diverge by 0.7 log units, despite the similar hydrophobicity parameters of the two cations (Py8-4Me and IM18). Similar, but smaller deviations are found for the $(C_2F_5SO_2)_2N$ salts of the same cations. It would be tempting to attribute these differences to H-bonding between the hydrogen in 2-position of the imidazolium ring and the sulfoxy groups of the bis(perfluoroalkylsulfonyl)imides in the pure ionic liquid, leading to smaller water solubilities of the imidazolium bis(perfluoroalkylsulfonyl)imides than predicted by their cation hydrophobicities. However, the size of the intralaboratory variability of the data as apparent in Table 5 in the Appendix does not really allow for such a conclusion, given that these data were generated by different groups.

In Table 4, the statistical parameters resulting from fitting this model to two sets of data are given. When modeling only the water solubility data from our labs (UFT only), the RSE was a little smaller than in the model for all solubility data (Full). This is not really surprising, as the equilibration procedure can have an impact on the results. The fact that the slope parameter m approximates the value expected according to the theoretical considerations is a striking evidence that both partitioning processes (ionic liquid/water equilibrium and cation partitioning in the HPLC column) are governed by the same cation property, even though the second phase is an ionic liquid in the one case, and a hydrophobic interphase at the surface of the chemically modified silica particles in the other case. The most plausible explanation is indeed to assume that this common property is their activity coefficient in aqueous systems with the pure ionic liquid as the reference state.

Figure 2. Plot of water solubility against $\log k_{0,c}$ of the cation of various ionic liquids that form a second phase with water at room temperature. To the left, only water solubility data generated in our labs are presented, to the right, water solubility data from the peer reviewed literature are included. Regression lines for constant anions have identical slopes $-m$ as defined by model equation 1. Only data for anions with solubility data for at least two different cations are plotted.



For the UFT only model, the slope m is 0.97, which is within the expected uncertainty for such a model. The larger deviation of m from unity in the full model can partly be explained by a large scatter of experimental values for different 1-octyl-3-methylimidazolium ionic liquids. The lower values better fit the theory, which might be caused by a disturbance of the chosen analytical method by the formation of microdroplets, which is also a known problem in the determination of 1-octanol/water partition coefficients.

The anion constants c_a in Table 4 can be seen as hydrophobicity measures, complementary to the $\log k_{0,c}$ values for the cations. The fact that many of these constants are derived from data for only one ionic liquid explains the very high coefficient of determination R^2 for both models. It is worthy to note that the anion constants in Table 4 for the $[PF_6]^-$, $[(CF_3SO_2)_2N]^-$, and $[(CF_3SO_2)_3C]^-$ anions are very similar, regardless if only the UFT data are employed in the model building, or if they are supplemented by the more abundant and completely independent literature data.

Table 4. Statistical parameters of the model described in equation 1 applied to only our own water solubility data at 293.15 K (UFT data only), and the complete water solubility dataset for 293.15 ± 5 K (Full dataset) given in Table 3. Numbers in parentheses after the anion constants are the number of data points for each anion that the constant is based on. n is the overall number of data points in the model, df is the number of statistical degrees of freedom, R^2 is the fraction of the variability in water solubility explained by the model, and RSE is the standard error of the residuals. Note that data points for anions that are only present in one IL have a zero residual, giving a favorable bias to R^2 and RSE. The anion acronym (6-2Et)2SS stands for bis(2-ethylhexyl)sulfosuccinate.

Parameter	UFT data only	Full dataset
m	0.97	0.881
$c_{(6\text{-}2Et)2SS}$		0.521 (1)
c_{BF4}		-1.268 (2)
c_{CF3SO3}		-1.343 (1)
$c_{C(CN)3}$		-1.642 (3)
c_{PhBF3}		-1.853 (1)
$c_{B(CN)4}$		-2.264 (1)
c_{PF6}	-2.178 (3)	-2.28 (18)
$c_{(C4F9)SO3}$		-2.61 (4)
$c_{(CF3SO2)2N}$	-2.868 (8)	-2.911 (50)
c_{AsF6}		-3.165 (1)
$c_{(C2F5SO2)2N}$		-3.363 (2)
$c_{(CF3SO2)3C}$	-3.841 (1)	-3.902 (2)
$c_{(C2F5)3PF3}$	-4.803 (2)	-4.883 (2)
n	14	88
df	9	74
R^2	0.999	0.998
RSE	0.157	0.16

A more direct determination of the anion hydrophobicity using an HPLC based method similar to the method of cation hydrophobicity determination used here was attempted. So far, the results have been found to be unreliable, presumably because of the limited suitability of the chromatographic partitioning system used.

The correlations presented here confound the validity of the $\log k_{0,c}$ values as derived from HPLC as a measure of the tendency of a cation to evade aqueous phases. Previously, we have shown that the cytotoxicity of ionic liquids can be described by the cation $\log k_{0,c}$ with an RSE value of 0.47 [14]. The much larger RSE in that former study can be attributed to a) the fact that several anions were included in

one correlation, b) to the inclusion of the large aromatic quinolinium cations in the earlier study, and c) to the fact that biological test systems generally yield higher variabilities than physicochemical systems.

The applicability domain of the model for predicting water solubilities from this model is naturally restricted by the availability of $\log k_{0,c}$ values (see [16] and this study) and anion constants (Table 4). The accuracy of $\log k_{0,c}$ values is best for $0 < \log k_{0,c} < 4$, because of limitations of the determination method. In this interval, the uncertainty is estimated to be less than 0.1 log units. The estimated standard error of predictions of water solubility in this interval is 0.2 log units for the seven anions shown on the right side of Figure 2 (model for selected data). For the anions with only one data point, we advise to only extrapolate to ionic liquids with cations that have a $\log k_{0,c}$ that does not deviate more than 1 log unit from the $\log k_{0,c}$ of the given data point.

For the convenience of the reader, the water solubilities estimated by the full solubility model are listed together with the experimental data in Table 5 in the Appendix. Besides the explanations given above, no systematic deviations from the model were noted. In general, the database for the bis(trifluoromethylsulfonyl) imide ionic liquids is quite extensive, and there is a very good agreement between the majority of literature sources where more than one value was found for the same ionic liquid. However, there are several outlying data points e.g. for IM18 bis(trifluoromethylsulfonyl)imide, that can only be explained by a considerable interlaboratory variability.

The approach taken here is in some ways similar to the one taken by Kakiuchi and co-workers, explaining the water solubility of ionic liquids in terms of their ion transfer potentials between ionic liquid and water [23], for which the ion transfer potentials between nitrobenzene and water are proposed as an approximation [33]. This group provided experimental evidence for the assumption that the ion transfer potentials between ionic liquids and water for the hydrophobic ions is independent of the concentration of hydrophilic ions like Na, K and Cl in the aqueous phase. This can be taken as a corroboration of our assumption, that the activity coefficients of the hydrophobic cations are similar in water as in the mobile phase of our liquid chromatographic system (Assumption 7 in the supporting information). However, the reliability of prediction methods for the water solubility of ionic liquids based on such theories is limited by the quantity and precision of experimental data.

5. Conclusions

A new pathway to the approximate quantification of aqueous ion solvation is shown, making use of the relative weakness of interactions between ionic liquid ions as compared to their hydrophobicities. We believe that the excess free energies of IL solution in aqueous phases that can be estimated from $\log k_{0,c}$ and c_A values presented here are a valuable tool to quantitatively predict equilibrium concentrations of ionic liquids in aqueous phases, contributing to the toolbox necessary for a molecular design of ionic liquid applications as well as to a more thorough understanding of their partitioning behavior in general.

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Appendix

Table 5. Overview of experimental (superscript exp) and estimated (superscript est) water solubility data, sorted by the estimated values. $\Delta_{\mathrm{est,exp}}$ is the difference between estimated and observed values on a log scale. The model based on all suitable data was used for the estimation.

Cation[a]	Anion[b]	$\log_{10} x_{\mathrm{IL,w}}^{\mathrm{sat,exp}}$		$\log_{10} x_{\mathrm{IL,w}}^{\mathrm{sat,est}}$	$\Delta_{\mathrm{est,exp}}$	Temperature [K]
IM16	$(C_2F_5)_3PF_3$	-5.93		-5.98	-0.05	293.15
Pyr14	$(C_2F_5)_3PF_3$	-5.43		-5.39	0.04	293.15
Py8-4Me	$(C_2F_5SO_2)_2N$	-5.4	[21]	-5.11	0.29	298.15
IM16	$(CF_3SO_2)_3C$	-5.04		-4.99	0.05	293.15
IM18	$(C_2F_5SO_2)_2N$	-4.7	[23]	-4.99	-0.29	298
Py8-4Me	AsF_6	-4.91	[21]	-4.91	0	298.15
Py8-4Me	$(CF_3SO_2)_2N$	-5.09	[21]	-4.66	0.43	298.15
Pyr18	$(CF_3SO_2)_2N$	-4.71	[22]	-4.56	0.15	298
IM18	$(CF_3SO_2)_2N$	-4.5	[25]	-4.54	-0.04	288.15
IM18	$(CF_3SO_2)_2N$	-4.59		-4.54	0.05	293.15
IM18	$(CF_3SO_2)_2N$	-4.1	[27]	-4.54	-0.44	293.15
IM18	$(CF_3SO_2)_2N$	-4.49	[25]	-4.54	-0.05	293.15
IM18	$(CF_3SO_2)_2N$	-4.14	[24]	-4.54	-0.4	296.5
IM18	$(CF_3SO_2)_2N$	-4.1	[26]	-4.54	-0.44	298
IM18	$(CF_3SO_2)_2N$	-4.6	[23]	-4.54	0.06	298
IM18	$(CF_3SO_2)_2N$	-4.47	[25]	-4.54	-0.07	298.15
Py6-4NMe2	$(CF_3SO_2)_2N$	-4.53		-4.5	0.03	293.15
Py6-4NMe2	$(CF_3SO_2)_2N$	-4.53	[24]	-4.5	0.03	296.5
IM14	$(CF_3SO_2)_3C$	-4.44	[24]	-4.49	-0.05	296.5
Py8-4Me	$(C_4F_9)SO_3$	-4.63	[21]	-4.35	0.28	298.15
IM17	$(CF_3SO_2)_2N$	-4.31	[25]	-4.29	0.02	288.15
IM17	$(CF_3SO_2)_2N$	-4.3	[25]	-4.29	0.01	293.15
IM17	$(CF_3SO_2)_2N$	-4.29	[25]	-4.29	0	298.15
IM18	$(C_4F_9)SO_3$	-4.23	[26]	-4.24	-0.01	298
IM16-2Me	$(CF_3SO_2)_2N$	-4.15	[24]	-4.12	0.03	296.5
IM16	$(CF_3SO_2)_2N$	-4.05	[25]	-4	0.05	288.15

Table 5. Cont.

Cation[a]	Anion[b]	$\log_{10} x_{IL,w}^{sat,exp}$		$\log_{10} x_{IL,w}^{sat,est}$	$\Delta_{est,exp}$	Temperature [K]
IM16	$(CF_3SO_2)_2N$	-4.18		-4	0.18	293.15
IM16	$(CF_3SO_2)_2N$	-3.86	[27]	-4	-0.14	293.15
IM16	$(CF_3SO_2)_2N$	-4.05	[25]	-4	0.05	293.15
IM16	$(CF_3SO_2)_2N$	-4.03	[24]	-4	0.03	296.5
IM16	$(CF_3SO_2)_2N$	-4.02	[25]	-4	0.02	298.15
Pyr16	$(CF_3SO_2)_2N$	-4.12		-3.94	0.18	293.15
IM18	PF_6	-3.95	[28]	-3.91	0.04	288.15
IM18	PF_6	-3.92	[28]	-3.91	0.01	293.15
IM18	PF_6	-3.93		-3.91	0.02	293.15
IM18	PF_6	-3.46	[30]	-3.91	-0.45	295
IM18	PF_6	-3.9	[28]	-3.91	-0.01	298.15
IM15	$(CF_3SO_2)_2N$	-3.74	[25]	-3.72	0.02	288.15
IM15	$(CF_3SO_2)_2N$	-3.73	[25]	-3.72	0.01	293.15
IM15	$(CF_3SO_2)_2N$	-3.71	[25]	-3.72	-0.01	298.15
Py8-4Me	$PhBF_3$	-3.6	[21]	-3.6	0	298.15
Py4-3Me	$(CF_3SO_2)_2N$	-3.7	[24]	-3.55	0.15	296.5
Py4-4Me	$(CF_3SO_2)_2N$	-3.69	[26]	-3.55	0.14	298
Pip14	$(CF_3SO_2)_2N$	-3.78	[22]	-3.51	0.27	298
IM14	$(CF_3SO_2)_2N$	-3.54	[25]	-3.5	0.04	288.15
IM14	$(CF_3SO_2)_2N$	-3.5		-3.5	0	293.15
IM14	$(CF_3SO_2)_2N$	-3.53	[25]	-3.5	0.03	293.15
IM14	$(CF_3SO_2)_2N$	-3.46	[27]	-3.5	-0.04	293.15
IM14	$(CF_3SO_2)_2N$	-3.49	[29]	-3.5	-0.01	294.15
IM14	$(CF_3SO_2)_2N$	-3.5	[24]	-3.5	0	296.5
IM14	$(CF_3SO_2)_2N$	-3.51	[26]	-3.5	0.01	298
IM14	$(CF_3SO_2)_2N$	-3.51	[25]	-3.5	0.01	298.15
Pyr14	$(CF_3SO_2)_2N$	-3.57		-3.41	0.16	293.15
Pyr14	$(CF_3SO_2)_2N$	-3.59	[22]	-3.41	0.18	298
IM16	PF_6	-3.45	[28]	-3.37	0.08	288.15
IM16	PF_6	-3.35		-3.37	-0.02	293.15
IM16	PF_6	-3.41	[28]	-3.37	0.04	293.15
IM16	PF_6	-3.36	[28]	-3.37	-0.01	298.15
IM13	$(CF_3SO_2)_2N$	-3.29	[25]	-3.28	0.01	288.15
IM13	$(CF_3SO_2)_2N$	-3.28	[25]	-3.28	0	293.15
IM13	$(CF_3SO_2)_2N$	-3.27	[25]	-3.28	-0.01	298.15
Py4-4Me	$(C_4F_9)SO_3$	-3.03	[26]	-3.25	-0.22	298
IM14	$(C_4F_9)SO_3$	-3.15	[26]	-3.2	-0.05	298

Table 5. Cont.

Cation[a]	Anion[b]	$\log_{10} x_{IL,w}^{sat,exp}$		$\log_{10} x_{IL,w}^{sat,est}$	$\Delta_{est,exp}$	Temperature [K]
IM12	$(CF_3SO_2)_2N$	-3.12	[25]	-3.1	0.02	288.15
IM12	$(CF_3SO_2)_2N$	-3.08	[27]	-3.1	-0.02	293.15
IM12	$(CF_3SO_2)_2N$	-3.1	[25]	-3.1	0	293.15
IM12	$(CF_3SO_2)_2N$	-3.1	[24]	-3.1	0	296.5
IM12	$(CF_3SO_2)_2N$	-3.08	[25]	-3.1	-0.02	298.15
Py8-4Me	CF_3SO_3	-3.09	[21]	-3.09	0	298.15
Mor11O2	$(CF_3SO_2)_2N$	-3.19		-3.06	0.13	293.15
Py8-4Me	BF_4	-2.98	[21]	-3.01	-0.03	298.15
IM18	BF_4	-2.93	[30]	-2.9	0.03	295
IM14	PF_6	-3	[28]	-2.87	0.13	288.15
IM14	PF_6	-2.8	[27]	-2.87	-0.07	293.15
IM14	PF_6	-2.9		-2.87	0.03	293.15
IM14	PF_6	-2.96	[28]	-2.87	0.09	293.15
IM14	PF_6	-2.93	[31]	-2.87	0.06	294
IM14	PF_6	-2.87	[29]	-2.87	0	294.15
IM14	PF_6	-2.89	[30]	-2.87	0.02	295
IM14	PF_6	-2.9	[24]	-2.87	0.03	296.5
IM14	PF_6	-2.92	[28]	-2.87	0.05	298.15
Py3OH	$(CF_3SO_2)_2N$	-2.43		-2.83	-0.4	293.15
IM12OH	$(CF_3SO_2)_2N$	-2.34	[24]	-2.66	-0.32	296.5
IM12	$B(CN)_4$	-2.46	[24]	-2.46	0	296.5
N4444	(6-2Et)2SS	-1.52	[32]	-1.52	0	298

[a] Cation acronyms explained in Table 1

[b] (6-2Et)-2SS = bis(2-ethylhexyl)sulfosuccinate

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B Selected further publications

B.1 Progress in evaluation of risk potential of ionic liquids - basis for an eco-design of sustainable products

Bernd Jastorff, Kerstin Mölter, Peter Behrend, Ulrike Bottin-Weber, Juliane Filser, Anna Heimers, Bernd Ondruschka, Johannes Ranke*, Maike Schaefer, Heike Schröder, Annegret Stark, Piotr Stepnowski, Frauke Stock, Reinhold Störmann, Stefan Stolte, Urs Welz-Biermann, Susanne Ziegert, Jorg Thöming

Green Chemistry **7** (5) 362-372

Motivated by the prevailing need for a sustainable development and taking the principles of Green Chemistry as a starting point, the present paper describes new and updated findings regarding a sustainable product design for ionic liquids. The focus is on environmental risk. Nevertheless, cytotoxicity testing and first indicative results from a genotoxicity study extend present knowledge also with regard to possible effects on humans. The structural variability of commercially available ionic liquids as well as the abundance of theoretically accessible ionic liquids is illustrated and the consequences for an integrated risk assessment accompanying the development process are discussed. The side chain effect on toxicity for imidazolium type ionic liquids was confounded by more complex biological testing. Also, an influence of an anion on cytotoxicity is shown for the first time. Testing of presumed metabolites of the imidazolium type cations showed a significantly lower biological activity in cytotoxicity studies than their parent compounds. The importance of a purity assessment for ionic liquids is pointed out and a collection of methods that is believed to be adequate is presented. In addition to risk analysis, the use of life cycle analysis for the multi-objective problem of designing ionic liquids is sketched and an eco-design scheme for ionic liquids is proposed. In conclusion, the paper illustrates the complex nature of the development processes ionic liquids are currently undergoing and provides guidance on which aspects have to be kept in mind.

doi: 10.1039/b418518h

B.2 Thinking in structure-activity relationships — a way forward towards sustainable chemistry

Bernd Jastorff, Reinhold Störmann, and Johannes Ranke*

CLEAN **35** (5) 399-405

Thinking in structure-activity relationships (T-SAR) is presented as an approach to a systematic collection and networking of knowledge and hypotheses regarding chemical structures. T-SAR can be accompanied by quantitative correlation studies commonly referred to as quantitative structure-activity relationships (QSAR) or linear free energy relationships (LFER). Some important limitations of these quantitative approaches are illustrated using the examples of biopartitioning and equilibrium partitioning in general. A systematic scheme for the qualitative analysis of a structural formula in seventeen steps is shown, covering stereochemistry, molecular interaction potentials, and reactivity. As an example for the application of T-SAR, contributions to the molecular design of ionic liquids are described.

doi: 10.1002/clen.200720018

B.3 Reconsidering environmental effects assessment of chemicals: Proposal for a dynamic testing strategy

Marion Junghans, Maike Schaefer, Wiebke Drost, Enken Hassold, Frauke Stock, Matthias Dünne, Tanja Juffernholz, Wiebke Meyer, and Johannes Ranke*

Basic and Applied Ecology **9** (4) 356-364

Certain substances may be hazardous to ecosystems. To be able to preserve the structures and functions of ecosystems, knowledge is required to qualify and quantify such hazards. To this end, biotests are indispensable tools. For the development and/or choice of biotests, special attention has to be drawn to conflicts between scientific demands and practical constraints. From a purely scientific point of view, experiments should be designed to maximise the ecological relevance of the obtained results. However, this often collides with the limited resources (budget, time, manpower) available. Furthermore, societal issues (e.g. animal welfare) have to be taken into account. Thus, it is necessary to develop a scientifically sound testing approach that avoids unnecessary animal testing, keeps the costs low, and can be performed within a short timeframe. The different perspectives of ecology, environmental toxicology, and environmental chemistry should be integrated into a balanced ecotoxicological approach. Accordingly, we propose a dynamic testing strategy, which is adapted to the substance (or substance group) in question and its mode(s) of action.

doi: 10.1016/j.baae.2007.08.011

B.4 Purity specification methods for ionic liquids

Annegret Stark*, Peter Behrend, Oliver Braun, Anja Müller, Johannes Ranke*, Bernd Ondruschka, and Bernd Jastorff

Green Chemistry **10** (11) 1152-1161

In the last decade, ionic liquids have shown great promise in a plethora of applications. However, little attention has been paid to the characterisation of the purity of these fluids, which has ultimately led to non-reproducible data in the literature. In order to facilitate specification of ionic liquids, a number of analytical protocols with their limits of detection (where available) have been compiled, including methods of other authors. In particular, quantitative methods have been developed and summarised for the determination of the total ionic liquid content, residual unreacted ionic liquid starting material and by-products (amines, alkylating agents, inorganic halides), solvents from extraction procedures and water, in addition to decomposition products and total volatiles.

doi: 10.1039/b808532c

B.5 Selected Conference Posters

Ranke J, Jastorff B (1999) Chemicals for tomorrow. In: Proceedings of the OECD workshop on sustainable chemistry, 15-17 October 1998 in Venice, Italy. OECD Environmental Health and Safety Publications Series on Risk Management No. 10.

Ranke J (2004) Joint bioaccumulation and joint biological activity as ecotoxicological risk indicators. Workshop "Internal Exposure - linking bioavailability to effects", August 22-27, 2004, Monte Verità, TI, Switzerland

Chemicals for tomorrow

J. Ranke, B. Jastorff

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THESIS I

The **choice of products** is generally dictated by ease of obtainment and technological aptitude. In the case of chemical products legal compliance and public acceptance are especially sensible parameters for their sustainability. In order to assure legal compliance and public acceptance on the long term anyone participating in the choice of chemicals must rely on information about their inherent risks to the biosphere.

THESIS II

A **transfer of knowledge** from pharmazeutical and agricultural chemistry is considered to be capable of improving qualitative and quantitative understanding of Structure-Activity-Relationships SAR. The cultivation of knowledge about fate and effects of chemicals is crucial for a sustainable business process.

THESIS III

Considering the significant difficulties in conducting a scientifically sound risk assessment for a specific application emphasis has to be put on methods for **low-expenditure risk analysis** resulting in indicators representing persistence, spatial range, bioaccumulation, biological activity and data limitation.

QUESTION I

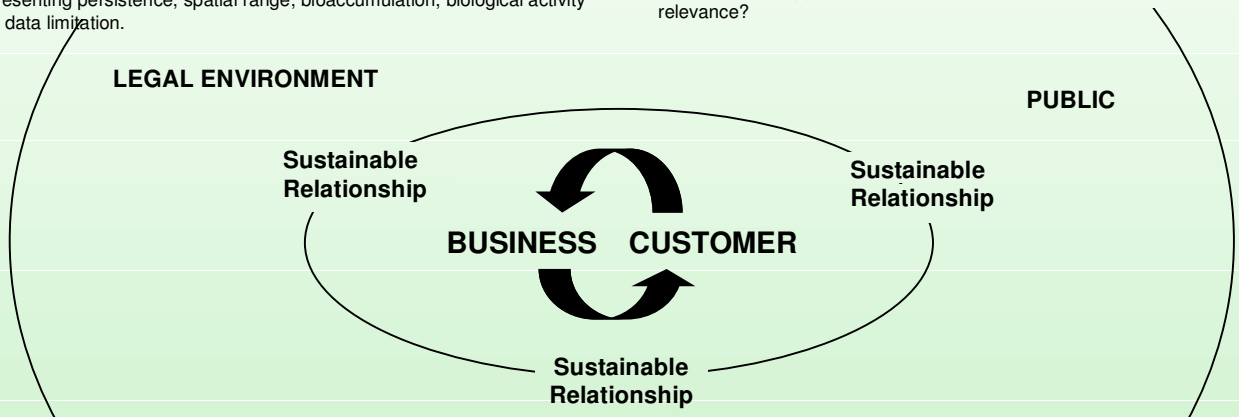
What are the **values** that we see in the surrounding biosphere? Do we want to protect it because of its functional value that can be shown or do we believe that there are inherent values that we can´t perceive through a functional perspective?

QUESTION II

What are the consequences that result from the **complexity** of the biosphere? Since abundance of chemical receptors in biological systems and amount of relationships between the organisms make predictions of effects on the basis of deterministic models seem impossible, are there alternative concepts of evaluation?

QUESTION III

How can we overcome the **reductionist scientific approach** in the prediction of effects without sacrificing fundamental principles of science such as rationality, transparency and universal validity? Is it scientifically acceptable to support evaluations on a weak factual basis, but with high relevance?



SOME THOUGHTS ON RISK INDICATORS

Persistence, defined by the global half-life of a chemical impulse, is traditionally an important argument in the evaluation of chemicals. It has to be pointed out that an estimated global half-life is far more meaningful than any half-life calculated from a degradation rate in a single environmental compound.

Spatial range as the 95%-quantile of the spatial distribution of expositions due to specific release of a chemical is a second exposure-based indicator that can be estimated by measured exposition data or by applying multi-compartment-models.

A further exposure-based indicator might be the fraction of the exposure to be found in organisms. Exact definition and development of methods for the determination of such an **organismic fraction** are yet to be carried through.

Due to the ongoing development of biological and ecological testing systems there is a large amount of often inhomogeneous data about effects of chemicals on organisms, organs, cells and cellular structures. Flexible aggregation of this data to an indicator for **biological activity** is desirable.

Data limitation is an important issue in the evaluation of ecological risks. The qualitative and quantitative limitations of available data necessary to determine the above indicators are an important measure of the uncertainty about the effects caused by the application of a chemical. Regarding ecosystem health as well as regarding human health it is true that every substance is toxic at some exposure/dose. Ignorance of the effect concentrations/doses of chemicals to be handled must be regarded as an indicator of risk.

RESEARCH AIMS

- Evaluation of performance and reliability of computer-based expert systems designed to predict fate- and effect- data
- Aggregating information from bioassays
- Case-studies for replacement chemicals
- Prediction of metabolites and analysis of their risks
- Cooperation with jurisprudence
- Considering the needs of small and medium size companies for risk analysis
- Transdisciplinary evaluation of risks

INTERDISCIPLINARY EDUCATION

- Undergraduate courses:
  „SAR of organic compounds“
  „The chemical logic of natural compounds“
- Post-graduate courses:
  „Ecotoxicology and risk assessment“

LITERATURE

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Joint bioaccumulation and joint biological activity as ecotoxicological risk indicators

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Introduction

Bioaccumulation is often used as an indicator of ecotoxicological risk. Within the concept of ecotoxicological risk profiles [1, 2, 3], this indicator is complemented by the risk indicators release R, spatiotemporal range S, biological activity B and uncertainty U.

Such a use of five indicators covers the whole pathway relevant for the decisions on ecotoxicological risks, starting from information about the entry of the substance into the environment up to reflexive information about the quality of the evaluation (Figure 1).

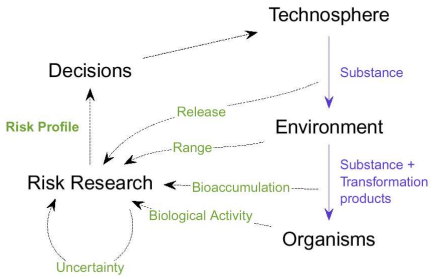

Figure 1: The information cycle of risk management along the pathway of environmental chemicals

While the release indicator R only deals with the estimated global release rate I of the *original* substance,

$$\mathsf{R} \propto \log_{10} I \tag{1}$$

the spatiotemporal range S includes *transformation products*. It can be perceived as the total amount of the substance and its relevant transformation products in the environment in steady state n_{env}, divided by its release rate, or, in other words, the joint residence time t_{joint} of the substance and its relevant transformation products within a model of the global environment (cp [4]).

$$\mathsf{S} \propto \log_{10} \frac{n_{\mathrm{env}}}{I} = \log_{10} t_{\mathrm{joint}} \tag{2}$$

Strictly following this train of thought, the bioaccumulation indicator B is defined as the quotient of the total amount of the substance including relevant transformation products present in organisms in steady state n_{bio}, divided by the total amount in the environment n_{env} as defined above.

$$\mathsf{B} \propto \log_{10} \frac{n_{\mathrm{bio}}}{n_{\mathrm{env}}} \tag{3}$$

Perceived in this manner, bioaccumulation as a risk indicator strongly depends on transformation reactions of the original substance as well as on the partitioning, uptake and clearance of transformation products. Only by virtue of this definition, highly bioaccumulating transformation products will directly contribute to the risk caused by the release of the original substance and substances with persistent metabolites and a correspondingly high spatiotemporal range will be judged by a "fair" metric of their bioaccumulation.

Taking it one step further, the indicator biological activity A should indicate the biological effects resulting from these substances present within the organisms, i.e. resulting from their total internal exposure.

Methodological approach

In this contribution, mathematical formulae for the estimation of thusly defined indicators are presented. Strategies for assessing biological activity from simultaneous measurements of biological effects and internal exposure are given special attention.

Relevance of transformation products

The question which of the known or suspected transformation products should be included in an assessment of joint persistence [4], secondary persistence [5] or spatiotemporal range [2], has not been answered in a general manner, so it is up to the risk researcher to judge which of them are regarded irrelevant for the risk assessment.

Modelling joint bioaccumulation

For the evaluation of the spatiotemporal range of the substance according to the definition given above (eqation 3), a fate model of the form

$$\dot{\mathbf{n}} = f(\mathbf{n}) + \mathbf{S} \tag{4}$$

can be defined, where $\mathbf{n}$ is a vector of the number of moles of each substance i in each model compartment j, therefore containing i times j elements. $f(\mathbf{n})$ contains descriptions of the change in number of moles in each compartment in dependence of the amount of every substance in every compartment. $\mathbf{S}$ is the source term, which has to be constant over time for a steady state solution.

Recently, not only fish biomass but also vegetation is being included in multimedia fate models, so the number of moles terms of equation 3 could be directly derived from steady state solutions of such models.

However, it is often unrealistic to assume that environmental loads will reach steady state concentrations within any period of interest for the decision-maker. Therefore, it is suggested here to use a numerical solution of the model defined according to equation 4 to estimate $\mathbf{n}(t)$ and to estimate the joint bioaccumulation according to equation 3 from $\mathbf{n}(t = 100\mathrm{y})$, assuming a realistic $\mathbf{n}(0)$ and a realistic source term $\mathbf{S}$ according to present knowledge.

This procedure for the evaluation of the risk indicator B is roughly equivalent to weighting the bioconcentration factors for the different compartments with the fractions of the substance present in them, but in addition allows for the inclusion of transformation products in favor of estimating a joint bioaccumulation.

Joint biological activity

Recently, increased attention has been focused on internal effect concentrations. Reasons for this are lower variabilities in internal effect concentrations as compared to external effect concentrations and the possiblity to combine biomimetic extraction for the estimation of total body residues (TBR) of complex contaminant mixtures with internal effect concentrations for the risk assessment of complex mixtures as e.g. effluents (compare e.g. [6]).

In the context of ecotoxicological risk profiles, internal effect concentrations have been chosen as measures for biological activity since they are much more independent of bioaccumulation measures than external effect concentrations.

The problem for a joint assessment of the biological activity of a substance together with its transformation products is that information about toxic effects not only for different biological species (living in different ecological contexts) has to be integrated, but also information for different chemical substances.

One conceptually possible solution of this dilemma is to calculate a weighted average of all internal effect concentrations with weights according to the fraction of the respective substance of the overall mass and according to the relevance of the species with regard to the predicted/known distribution pattern across the environmental media. This approach would be an extension of the one used in the original definition of the ecotoxicological risk profiles [2, 3].

Another approach newly presented here is to build an average of the critical biomasses m^{crit} which are defined here as the biomass needed to dilute one mole of a chemical to a degree that no adverse chronic effect can be observed.

$$\mathsf{A} \propto \sum_{k,j} f_{k,j} \cdot m^{\mathrm{crit}}_{k,j} \tag{5}$$

In the above definition, k is an index over all biomass compartments of the model, j is the indicator over the substances (original plus transformation products), $f_{k,j}$ is the fraction of substance j in biomass compartment k of the total accumulated number of moles n_{bio} and $m^{\mathrm{crit}}_{k,j}$ is the critical biomass volume of substance j in biomass type k as defined above. $V^{\mathrm{crit}}_{k,j}$ values are specific for each type of biomass which is considered by the fate model.

This definition is inspired by the life cycle assessment (LCA) method of critical volumes, where the effects of emissions to the environment are summed up using their critical volumes as weights, calculated from limit values for their concentrations in different environmental media.

It also draws some validity on the assumption that the bioaccumulated mass of substances with equal mode of action can just be added up regardless of their exact identity, as put forward in [6].

Concluding remarks

The main point of generating ecotoxicological risk profiles rather than estimating risk ratios is the need for a method which can cope with partial and uncertain information without producing misleading results. The method is meant to inform decision-makers during the development of chemical products and processes as well as for regulators concerned with the global environmental risk of the release of chemicals.

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