Scientific Committee on Emerging and Newly Identified Health Risks
SCENIHR

Scientific Committee on Consumer Safety
SCCS

Scientific Committee on Health and Environmental Risks
SCHER

Addressing the New Challenges for Risk Assessment

Discussion paper approved for public consultation in view of receiving feedback from stakeholders for its further development

The Inter-Committee Coordination Group approved this discussion paper for public consultation at its meeting of 8 October 2012

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

SCCS members

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Mohammad Chaudhry, Gisela Degen, Gerhard Eisenbrand, Corrado Galli, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Kai Savolainen, Jacqueline Van Engelen, Maria Vinardell, Rosemary Waring, Ian White

SCHER

Opinions on risks related to pollutants in the environmental media and other biological and physical factors or changing physical conditions which may have a negative impact on health and the environment, for example in relation to air quality, waters, waste and soils, as well as on life cycle environmental assessment. It shall also address health and safety issues related to the toxicity and eco-toxicity of biocides.

It may also address questions relating to examination of the toxicity and eco-toxicity of chemical, biochemical and biological compounds whose use may have harmful consequences for human health and the environment. In addition, the Committee will address questions relating to methodological aspect of the assessment of health and environmental risks of chemicals, including mixtures of chemicals, as necessary for providing sound and consistent advice in its own areas of competence as well as in order to contribute to the relevant issues in close cooperation with other European agencies.

SCHER members

Ursula Ackermann-Liebrich, Rolf Altenburger, Herman Autrup, Denis Bard, Stella Canna Michaelidou, Wolfgang Dekant, Pim De Voogt, Arielle Gard, Helmut Greim, Ari Hirvonen, Colin Janssen, Renate Kraetke, Jan Linders, Borut Peterlin, Jose Tarazona, Emanuela Testai, Marco Vighi

SCENIHR

This Committee deals with questions related to emerging or newly identified health and environmental risks and on broad, complex or multidisciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk assessment bodies. Examples of potential areas of activity include potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters and electronically controlled home environments), and methodologies for assessing new risks. It may also be invited to address risks related to public health determinants and non-transmissible diseases.

SCENIHR members

Anssi Auvinen, James Bridges, Kenneth Dawson, Wim De Jong, Philippe Hartemann, Arne Hensten, Peter Hoet, Thomas Jung, Mats-Olof Mattsson, Hannu Norppa, Jean-Marie Pagès, Ana Proykova, Eduardo Rodríguez-Farré, Klaus Schulze-Osthoff, Joachim Schüz, Mogens Thomsen, Theo Vermeire

Contact:

European Commission
DG Health & Consumers
Directorate D: Public Health Systems and Products
Unit D3 - Risk Assessment
Office: B232 08/015 B-1049 Brussels

Sanco-SCCS-Secretariat@ec.europa.eu

Sanco-SCHER-Secretariat@ec.europa.eu

Sanco-SCENIHR-Secretariat@ec.europa.eu

ISSN 1831-XXXXXXXX
doi:10.2772/XXXXXXX
ISBN 978-92-79-XX
ND-XXXXXX

The opinions, memoranda and discussion papers of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. These documents are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/policy/index_en.htm

ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this discussion paper. The members of the working group are:

Members of SCENIHR, SCHER, SCCS:

Herman Autrup, SCHER

Jürgen Angerer, SCCS

Jim Bridges, SCENIHR, Chair, Rapporteur (Human RA)

Peter Calow, SCHER

Qasim Chaudry, SCCS

Wolfgang Dekant, SCHER

Colin Janssen, SCHER

Mats-Olof Mattsson, SCENIHR

Emanuela Testai, SCHER

Marco Vighi, SCHER, Rapporteur (Ecological RA)

External experts:

Antonio Di Guardo, University of Insubria, Italy

Volker Grimm, Helmholtz Centre for Environmental Research, Germany

Robert Stones, Food and Environment Research Agency, UK

Dominique Lison, UCL, Belgium

Armin Wolf, Novartis, Germany

Colin Brown, University of York, UK

Frederik de Laender, Ghent University, Belgium

Joop Hermens, Utrecht University, The Netherlands

Ryszard Laskowski, Jagiellonian University, Krakow, Poland

Matthias Liess, UFZ, Leipzig, Germany

Dik van de Meent, RIVM, The Netherlands

Paul van den Brink, Alterra, Wageningen, The Netherlands

Nico van Straalen, VU University Amsterdam, The Netherlands

Discussion paper to be cited as:

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCHER(Scientific Committee on Health and Environmental Risks), SCCS (Scientific Committee on Consumer Safety), Preliminary report on Addressing the New Challenges for Risk Assessment, 8 October 2012

ABSTRACT

For a number of scientific and other reasons, the procedures currently used for both human and ecological risk assessment are anticipated to change substantially over the next few decades. However, the roadmaps for these changes are expected to be distinctly different for ecological and human health risk assessments.

ECOLOGICAL RISK ASSESSMENT

The approaches in current use for ecological risk assessment are likely to suffice for regulatory purposes as sufficiently protective for ecosystems. However they lack environmental realism. This entails high uncertainty on the actual consequences of environmental contaminations on the ecosystem structure and functions that has to be addressed by the application of uncertainty/safety/default factors.

The main challenge for ecological risk assessment is to develop tools that take account of the complexity of the potentially exposed ecosystems and enable assessment of site-specific effects.

Exposure considerations

- Verification and harmonization of physico-chemical data is necessary.
- Current models for fate prediction are better suited for apolar compounds. New and improved models are needed for polar and ionized chemicals and metals.
- Methods are needed to characterize the exposure to nanomaterials
- Criteria and protocols are required for obtaining and comparing monitoring data especially for evaluating the fate of chemical mixtures, including metabolites and breakdown products. Data at short temporal resolution (e.g. hours) are needed for developing/calibrating predictive approaches also in view of the rapid conversion of chemicals in some compartments of the ecosphere.

* New models are necessary for a number of purposes including:

- the development of realistic scenarios, especially to predict temporal and spatial variations as well as bioavailability of chemicals.

- Assessment of specific organism parameters to extend the applicability of bioaccumulation models in aquatic and terrestrial systems.

- Description of the food web path of chemicals, especially for terrestrial systems

Effects considerations

The Protection of high hierarchical levels of ecological organisation (communities, ecosystems) is the main goal of environmental protection. Mesocosm data and SSD are already a powerful tool for improving ecological realism of risk assessment. The usefulness of molecular approaches in ecological risk assessment remains to be established. They may be suitable as early warning systems.

Priorities for improvements are:

- The assessment of the effects of variable exposure due to space and time variability of chemical concentrations.
- The development of improved models to examine the vulnerability of aquatic and terrestrial ecosystems to different kinds of stressors, particularly for site-specific risk assessment.
- The improvement of knowledge on the interactions of toxicants with other environmental factors in natural ecosystems.

- The improvement of the application of trait-based ecological risk assessment.
- The development of ecological models capable to describe and predict direct and indirect effects of stress factors on structure and functions of ecosystems.
- A concerted action is needed to agree on standard scenarios, ecologically relevant test species and endpoints, acceptance criteria of ecological models, and to develop well-tested, flexible models.
- The increased complexity of the assessment would require statistically-based tools capable to quantitatively assess uncertainties and to improve the transparent use of these approaches.

HUMAN RISK ASSESSMENT

There is a trend/need to change the basis of risk assessment from the one based on standard tests to one that is centred on modes of action. A prerequisite for major advances is the development of improved databases to enable more appropriate test selection through advancement of in silico approaches – such as (Q)SAR and read-across. This will require fully validated databases for:

-Effects of chemicals in humans;

-Exposure information;

-Effects in animal models;

-Effects in *in vitro* models.

-Mode of action information

Exposure considerations

A paradigm shift is likely from a hazard-driven process to one that is exposure driven. Achieving this will require major improvements in the assessment of exposure to individual chemicals and groups of chemicals.

Priorities for improvement are:

-Advances in the identification and use of biomarkers for exposure.

-Wide availability of low cost personal monitors

-Better modelling of external and internal exposure.

Hazard considerations

Major changes are also needed in the identification and characterisation of hazards to humans. The development of alternatives to using laboratory animals for the identification and characterisation of hazardous properties of chemicals is a priority because of the political, ethical, and other pressures to reduce the use of laboratory animals for testing purposes. In investigations using laboratory animals, increasing importance should be directed to characterising the mode of action with less emphasis to endpoints based on histopathological criteria, body and organ weight, and blood chemistry.

Priorities for improvements are:

- The progressive replacement of *in vivo* laboratory animal tests by *in vitro* tests is critically dependent on the development of *in vitro* preparations that maintain the *in vivo* characteristics of various tissues and organs over long periods (weeks to months).

- New, more sensitive methods for characterising the effects of chemicals, in particular genomics, are likely to provide a very important tool for identifying modes of action which will increasingly become crucial for characterising the risks.

* Quantitative histochemistry and high content cell imaging will be important tools in linking biochemical changes to morphological (including histopathological) ones.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

As a consequence of advances in scientific knowledge as well as in modelling and measuring techniques, the procedures currently used for human and environmental risk assessment are required and anticipated to change substantially over the next few decades. However, roadmaps for these changes are expected to be distinctly different for ecological and human health risk assessments.

A. ECOLOGICAL RISK ASSESSMENT

The tradition of ecotoxicology is a few decades old and the development of ecological risk assessment is relatively recent. Indeed, the need for environmental protection was only recognised by the scientific and regulatory communities around the middle of the last century. In this relatively short period, the main objective was the development of simple tools, suitable to be applied successfully to provide answers to growing and pushing problems, even using relatively scarce information. The approaches currently used for ecological risk assessment represented the basis for the development of international regulations and are likely to be sufficiently protective for ecosystems.

The major drawback of these approaches is the lack of environmental realism. This leads to high uncertainty on the actual consequences of environmental contaminations on ecosystem structure and functions. This uncertainty is generally covered by the use of assessment factors in the risk assessment.

Some of the major issues at the origins of this high uncertainty are listed below:

- The variability of environmental scenarios that may strongly affect both exposure (environmental fate, bioavailability, etc.) and effects of toxic chemicals.
- The time and space variability of exposures.
- The interactions among the different stress factors (chemical, physical, etc.) that may affect ecosystem health and interact with toxicant effect.
- The interactions among individuals and populations in a biological community responsible for indirect ecological effects in the ecosystem.
- The species and site-specific vulnerability of populations and ecosystems.

Therefore, the major challenge for ecological risk assessment is the development of tools that can increase the ecological realism of exposure and effect assessment, taking into account the properties of potentially exposed ecosystems in risk characterisation and allowing the assessment of site-specific risk.

This report presents synthetically the major issues relevant for improving the realism of exposure and effect assessment and for reducing the uncertainty in ecological risk assessment, considering the characteristics of ecosystems potentially exposed. It describes both the processes that need to be better known and the tools to be used to achieve the desired result. It also evaluates the science behind them and the practical usefulness and applicability of the tools before identifying the needs for research.

Exposure assessment

While a number of tools are available to predict the fate of chemicals in several compartments, many uncertainties in exposure assessment remain and proper tools are still lacking at different levels.

- Sorption and bioavailability: while tools are available to assess sorption and bioavailability for non polar organic chemicals and metals; polar and ionized chemicals are generally soluble, but with highly variable solubility and solubility rates, making predictions sometimes difficult.

- Nanomaterials: very little is known to characterize the fate and exposure of these materials. However research (including EU projects) is ongoing.
- Better use of monitoring data: need for criteria and protocols for obtaining and comparing monitoring data in risk assessment, especially for evaluating the fate of chemical mixtures, including metabolites. Also data at short temporal resolution (e.g. hours) are missing and would be required for developing/calibrating predictive approaches. There is a need to collect and report monitoring data to explore spatial and temporal variability of concentrations and to assemble them in databases according to QA/QC criteria to be employed for model development, verification and validation.
- Need to verify and harmonize the physical-chemical data obtained in the REACH regulation according internationally recognized data quality requirements
- Improvement of modelling approaches**:**
    - Developing models for polar and ionized chemicals as well as for nanomaterials
    - Developing models capable to predict time and space variable concentrations in order to be compared to monitoring data and account for realistic exposure scenarios.
    - Developing realistic scenarios with a variation of environmental characteristics to reflect the ecological variability of conditions in the whole European situation
    - Obtain specific organism parameters to extend the applicability of bioaccumulation models in aquatic and terrestrial systems.
    - Developing models to describe the food web path of chemicals, especially for terrestrial systems, including the role of vegetation uptake in driving/regulating the input to the food chain and organic carbon cycle.

Effects assessment

To achieve the objective of protection of structure and functions of ecosystems, there is the need for a deeper knowledge of ecological processes and for the development of tools that may be useful to describe and predict them as also highlighted by the EU/SETAC workshop EPiF including 75 scientists from academia, industry and regulation (Liess et al 2005). Some issues already have sound scientific basis and may be practically applied, even if large margins for improvement still exist. In other cases, the present level of knowledge is not deep enough and suitable tools are not sufficiently developed for a practical application in risk assessment. These issues must be considered as relevant priorities for ecotoxicological research in the next future.

- Higher tier effect assessment. Mesocosm data and SSD are already used successfully in ecological risk assessment. Even if several improvements are possible (particularly for the development of more standardised methods capable of producing more reproducible results), they already represent a powerful tool for improving ecological realism of risk assessment. A relevant problem for their use in regulatory assessment is the improvement of transparency in the evaluation of the results, reducing the need for expert judgement. In particular, a priority for research is the development of statistically-based tools capable to quantitatively assess uncertainties and to improve the transparent use of these approaches.
- Effect assessment for complex exposure patterns. Accounting for time and space variability is a key issue for exposure assessment. For assessing the effects of such variable exposures, toxicokinetic/toxicodynamic (TK/TD) models seem to be the most suitable tool. The improvement of these models, and in particular their

experimental validation with organisms representative of aquatic and terrestrial biological communities, is a priority for research.

- Effect assessment at low hierarchical level. The investigation of parameters at the sub-individual level (e.g. gene expression, biochemistry) is widely applied in ecotoxicology. Omics tools can detect substance-specific effects at the low exposure concentrations prevailing in the environment. However, at present, the relationship between molecular effects and responses at higher hierarchical levels (population, community) is largely unknown. Considering that protecting high hierarchical level is the goal of environmental protection, the usefulness of molecular approaches in ecological risk assessment remains to be established. At present, they seem suitable as early warning systems which need to be calibrated against the safe levels needed to protect structure and functions of ecosystems. However, confounding parameters are preventing a clear link between biomarkers at subindividual level and exposure to chemicals. Therefore, their predictive capability is poor.
- Ecosystem vulnerability. The relevance of vulnerability evaluation for ecological systems is recognised, particularly for site-specific risk assessment. However, a practical application of the vulnerability concept in risk assessment requires tools able to express it in quantitative terms. Some have already been developed and proposed to quantify vulnerability to specific stress factors and in specific ecosystems (mainly rivers). Other preliminary proposals still require careful calibration and validation. For the practical use of the vulnerability concept in site-specific ecological risk assessment, the development, application and validation of methods capable of assessing the vulnerability of aquatic and terrestrial ecosystems to different kinds of stressors, is a priority for research.
- Endocrine disrupting effects. Endocrine disrupting chemicals (EDCs) may produce ecologically relevant effects affecting population dynamics. Present knowledge is mainly focused on vertebrates, particularly mammals, and little is known on endocrine systems of invertebrates. Considering the complexity of the issue and the relevance of endocrine disruption for ecosystem protection, the improvement of knowledge on endocrine systems in invertebrates and the development of procedures for assessing endocrine disrupting effects represent a priority for research.
- Indirect ecological effects. Indirect effects due to ecological interactions are a key issue for assessing actual consequences of stress factors at the ecosystem level. They can override direct effects and can mitigate but also exacerbate them. Experiments and community models have demonstrated the importance of indirect effects, but overall knowledge is still poor, particularly for use in risk assessment. The development of more comprehensive studies, based on experiments, inverse statistical modelling, and ecological modelling it is a key issue for assessing effects at ecosystem level and must be considered as a priority for research needs.
- Interactions with environmental factors. While the science behind the assessment of effects of several chemicals in combination is sound enough for proposing the introduction of mixture risk assessment in international regulations, the knowledge on the interactions of toxic chemicals with other potential stress factors is much less developed. In the recent literature, studies on the interactions of toxicants with other environmental factors have not received enough focus for ecological risk assessment. Considering the relevance of the issue, it must be considered a priority for future research needs.
- Trait-based risk assessment. Trait-based approaches represent a promising tool capable, in perspective, to complement taxonomically based assessments (difficult, time consuming and requiring taxonomic specialists) with functionally based assessment (more significant in ecological terms). At present, they

represent a tool for vulnerability analysis and for many other approaches relevant for ecological risk assessment. One of the bottlenecks for the development and application of the approach is the lack of data for the precise characterisation of suitable traits. This is particularly relevant in the cases requiring particular traits describing detailed anatomic characteristics as well as physiologic or metabolic patterns. The development of tools and databases for improving the application of trait-based ecological risk assessment represents a priority need for research.

- Ecological modelling. Ecological models, often in combination with individual-level effect models like toxicokinetic/toxicodynamic or DEB (dynamic-energy budget) models, are the most promising way to fully take into account "ecology" in risk assessment. Current modelling practice is too diverse and not transparent for regulatory risk assessment. However, good modelling practice is currently under development. A few models can already be used to address specific questions but still need to be assessed by regulators on a case-by-case basis. To make full use of the potential of ecological models, a concerted action similar to FOCUS for exposure models is needed to agree on standard scenarios, ecologically relevant test species and endpoints, acceptance criteria of ecological models, and to develop well-tested, flexible models that are both routinely used and improved. This is a very important priority for research but practical results for application in risk assessment are unlikely in the next few years.

Ecologically based risk characterisation

Once the relationships between varying exposure concentrations and consequent effects on populations and communities are clear and scientifically sound, dynamic exposure models and ecological models can be linked to study site-specific responses to chemicals. These relationships can be used to assemble exposure scenarios for a variety of environmental systems in order to be applied in the regulatory framework.

The development of new tools would reduce the uncertainties. However, suitable statistical and probabilistic approaches may represent effective tools for a better assessment of uncertainties. This will lead to a more scientifically sound assessement of the weight of evidence and to a more transparent risk characterisation.

B. Human-health risk assessment

The approach currently used is hazard driven with strong reliance on the use of laboratory inbred rat strains and to a lesser extent, inbred mouse strains as test species.

Over time, these tests have been increasingly standardised by the introduction of good laboratory practice and ICH or OECD test guidelines. Some *in vitro* tests, in particular for genotoxicity and topical effects have been added. Many of the tests in current use are written into legislative requirements for the approval of various types of products.

To address uncertainties due to the need for extrapolation when using data obtained in rats and mice to characterise effects that may occur in humans, conservative standard default values (also called assessment factors, uncertainty factors or default factors) have come into common use. There are both political and scientific reasons why a change in the way that human risk assessment is conducted. The primary changes proposed may be characterised as follows:

- A paradigm shift from a hazard driven process to one that is exposure-driven,
- A progressive reduction of tests using laboratory animals.

Exposure assessment

The quantification of exposure, both in individuals and in populations, is a prerequisite for the quantification of risk. Reliable data on exposure are needed to assess the probability of adverse effects of the stressor and to recognize specific risk factors such as occupation, life style, and social status. The dimensions of exposure include intensity, frequency, route, and duration; in addition, the nature, size, and makeup of the exposed population should be characterized.

Although a number of major advances in both chemical identification and quantitative analysis have been achieved, exposure assessment remains the weakest part of the majority of human risk assessments. There are a number of reasons for this:

- In the development of the current risk assessment methodology, improvements in exposure assessment have been given lower priority.
- Human exposure assessments often rely on assumptions on consumer behaviour, the estimation of which is associated with a number of biases.
- Investigations have tended to focus on single sources of exposure rather than on multiple sources which is the more common exposure situation
- Often exposure assessments have given insufficient attention to bio- and chemo-transformations

Advances in exposure assessment are crucial. The techniques that appear to be most promising to assess external exposure are:

Monitoring of personal exposure

In order to define the exposure scenarios, a better understanding of lifetime activities would be needed. Developments should be directed to obtaining:

- Estimates of both typical and high exposures in different age groups and the factors that most influence this, i.e. use pattern
- Information on trends in exposure over time to particular chemicals of 'concern', due to societal or behavioural changes.

The development of new monitoring techniques (e.g. personal monitors) will also make post marketing surveillance of exposure to air borne chemicals easier and cheaper. To assess airborne exposure to chemicals, especially in the workplace, a particularly desirable development would be the availability of low cost personal samplers to enable individual exposures to be assessed. The increasing availability of better absorbents and advances in technologies such as 'the laboratory on a chip' make this a realistic prospect. The main issues are to ensure proper prioritisation based on which groups of chemicals to measure and in which media. The availability of information provided within REACH would be essential for the prioritization and for the definition of appriopriate exposure scenarios. The main barrier will be the availability of low cost high throughput measuring devices able to measure accurately a wide range of chemicals and their transformation products.

Prediction of external exposure

There are a number of challenges for improving models of external exposure: one descends from the need of accounting for variability in space and time of environmental concentrations (including food). This would allow to better estimate human variability in exposure, especially at different stages of the life cycle. Other issues are related to the many uncertainties in the understanding of the behaviour of polar chemicals, nanomaterials, mixtures, as well as bioaccumulation in food of different origin. There is also a need for a harmonized approach in modelling strategies for different categories of chemicals, such as industrial substances and plant protection products.

Modelling of exposure will only represent the external dose, but combined with PBPK this information can be transformed into the internal dose.

Determination of internal exposure

The techniques to estimate internal exposure depend on information of external exposure but also require improved methods for determining absorption, distribution and excretion. It needs to be recognized that the development of *in vitro* systems requires appropriate methods to apply each chemical as well as uptake and metabolism systems that reflect those *in vivo*. Developments in PBPK modeling is likely to be an important tool by which the *in vitro* to *in vivo* interface can be improved.

Use of a tiered approach

A Tiered approach to the assessment of exposure to individual chemicals is recommended in which priorities for further work on individual chemicals are determined particularly by the estimated human exposure (nature, routes, levels, duration).

Hazard assessment

There is likely to be a continuing pressure to replace animal testing for risk assessment, prioritisation and classification by one or more of the following methods: non-testing methods, such as grouping and read-across, Thresholds of Toxicological Concern, exposure based waiving, and computational methods (SARs, QSARs, biokinetic modelling), *in vitro* tests, and optimised *in vivo* tests such as the Extended One-Generation Reproductive Toxicity Test. Since most of such alternative methods cannot be used as stand alone, it will be necessary to integrate them into a so-called integrated or intelligent testing strategy (ITS) based on Weight-of-Evidence methods integrating several of the above mentioned independent sources of information and information on mode or mechanisms of action. A shift is foreseen towards using more and more human data on biologically significant perturbations in key toxicity pathways, in such integrated testing strategies.

Key requirements for this are:

New in vitro methods

Requisites are:

- Establishment of in vitro preparations that preserve all the properties of their in vivo original source for prolonged periods of time;
- Means of reflecting in vivo toxicokinetics in vitro;
- Establishment of clear relationship between *in vitro* endpoints and adverse effects *in vivo*.

New endpoints

Sensitive measurement methods are needed to allow studies to be made at exposure levels that reflect likely human exposures. Omics technologies are likely to play progressively a key role.

Mode of action

Modes of action identify the biochemical pathways that link exposure to a chemical to immediate or eventual outcomes. Although studies of the mode of action are a focal point in the development of medicines and pesticides, this is not yet the case for industrial chemicals. Mode of action studies must become the central plank of a future risk assessment along with reliable and relevant exposure assessment. In considering promotion of the above technologies attention should be given to how they might facilitate advances in understanding modes of action and how the technologies could benefit from knowledge of modes of action. Mode of action information is also essential for the assessment/prediction of chemical interactions in mixtures.

A tiered approach

To enable the most effective use of resources and to limit the unnecessary use of animals a tiered approach to the assessment of hazards from exposure to individual stressors has been identified. Before conducting a hazard characterisation, information should be sought on previous studies on the stressor under examination.

Databases

The availability of comprehensive, validated and up to date databases is the essential foundation for the development of the new paradigm. Of the various needs that have been identified above, the most important are:

- Effects of various stressors in humans.
- Measurement/monitoring data on human exposure to various stressors.
- Extending the database that is a prerequisite for the TTC.
- The modes of action responsible for each type of adverse effect.

The development of fully validated databases will be essential for the advancement of (Q)SAR and read-across approaches in risk assessment.

Modelling integration

Exposure prediction should be implemented based upon an integrated (coupled) external (environmental fate, occupational exposure and food uptake) and internal (toxicokinetic, such as PBPK models) dynamic exposure model and bioindicators of effect. The integrated modelling approach, being quantitative, would allow to finetune the threshold of tolerable usage and emission of a chemical (including metabolites and same mode of action compounds) in a complex exposure situation

Risk characterization

Development and application of the paradigm will involve input from a new range of methods and tools. This will require a much greater dependence on scientific judgement in order to better assess the weight of evidence. For example, it will be essential to distinguish between changes which should be deemed as normal physiological changes to a stressor and a response that should be considered as adverse. This has major implications for the training and range of research experience of future risk assessors.

1. BACKGROUND

Risk assessment must be based on the best available scientific evidence. While the data base that supports risk assessments continues to expand and despite several challenges encountered, the general procedures have not changed significantly in the last two decades. Some current challenges discussed in two opinions on Harmonisation of Risk Assessment Procedures by the Scientific Steering Committee (SSC) (2000[1], 2003[2]) include access to data, exposure assessment and the explanation/ expression of findings.

Furthermore, there are a number of anticipated changes concerning both the nature and the interpretation of data available for risk assessment in the near future. Possible changes include the following:

1. Increasing restrictions on the use of animals for testing purposes in the EU and the need to develop and use alternative *in vitro* and *in silico* testing strategies and, possibly, appropriate modelling techniques.
2. The availability of data from new/rapidly advancing methodologies and the associated risk of information overload and lack of an effective process for the appropriate utilisation for risk assessment purposes.
3. Developments in mode of action research and its future use.
4. Developments of novel systems that may constitute new risks and may furthermore challenge traditional approaches used in Risk Assessment (e.g. products created by synthetic biology, 4th generation nanotechnologies)

2. TERMS OF REFERENCE

The SCENIHR, SCCS and SCHER are requested to carry out a comprehensive review of risk assessment procedures and new challenges for RA taking into account both fundamental and practical considerations (sampling, instrumentation, cost, analysis, etc.), and to provide a scientific discussion paper on the issue in co-operation, as appropriate, with external experts who are specialists in relevant new methodologies.

This group should consider the above and other relevant scientific issues relevant for a future framework for risk assessment and to propose a way forward. They should also consider the need to train future risk assessors in the understanding of these methodologies and their potential applications. Finally, the group should identify and prioritize areas for possible research funding.

It is proposed that the chairs of SCENIHR, SCHER and SCCS identify a small group of experts representing the different sectors and key disciplines.

[1] http://ec.europa.eu/food/fs/sc/ssc/out82_en.html

[2] http://ec.europa.eu/food/fs/sc/ssc/out355_en.pdf

3. GENERAL INTRODUCTION

3.1. Scope of the discussion paper

This discussion paper is centred on consideration of the scientific developments that are required to attain the desired major changes in human and ecological risk assessment. In general the term "environmental risk assessment" is used with the intention to also include the exposure of humans through the environment. In this discussion paper, the term "ecological risk assessment" is used with the intention not to include the route to humans and to focus on the environment itself. It is recognised that ethical and resource availability will also have an important influence on progress, but these aspects are outside the terms of reference.

Although humans and the environment are exposed to a combination of thousands of chemicals, usually only a limited number of them play a significant role, when assessing the adverse effects of real combined exposures. The main challenge is to identify which chemicals should be given priority for risk assessment and risk management, as the relevance is different for each individual and each ecosystem, and may also change with time.

Risk assessments are required by legislation for an ever increasing range of chemical, biological and physical agents and processes (termed stressors thereafter in this document). The focus in this discussion paper is on chemicals.

Risk assessment methodology requires continual review to ensure that the best available practice, based on sound current science, is being used. A number of additional factors make a thorough examination of current and potential future methods particularly timely:

- Public/political demands to reduce or abolish the use of animals for toxicity testing.
- The very large number of untested or inadequately tested chemicals and chemical products for which information is required on the hazards and risks involved in their use.
- Challenges posed by the need to assess more complex products and processes e.g. products of nanotechnologies, synthetic biology etc.
- The need to revisit approaches to issues such as the effects of exposure to combinations of chemicals and identification of vulnerable population groups and ecosystems.
- The question about the optimal use of the expected major increase in the information on commercialised substances and mixtures, following the new legal requirements for data generation and dissemination considered under the new EU legislative frame on chemicals safety (e.g. REACH and CLP Regulations).
- The development of education measures to ensure that there is sufficient high level expertise to enable the new risk assessment approaches to be applied.
- The need for dialogue with risk managers and for socio-economic analysis to ensure that the risk assessments are of practical value.

The purpose of this discussion paper is to try to identify how these factors are likely to influence the risk assessment methodology, for both humans and ecosystems, in the next few decades and what the implications of this are likely to be. The discussion paper is focussed on the risk assessment of chemicals, regardless of their use. However the conclusions of this assessment will have implications for the risk assessment for biological and physical stressors and for combinations of stressors. The report is intended to complement the discussion paper of the non-food scientific committees on improvements in risk assessment which focuses on improving the utility of risk assessments for risk

managers. Where relevant to future methodology the findings of this other report will be taken into account.

It is important to identify at the outset of this review what the vision is of a future risk assessment methodology and to chart a pathway or pathways that could lead to the achievement of this vision.

Taking into account the differences and the different meanings of challenges in human toxicology and ecotoxicology, after some general considerations (section 3.2), the overall discussion paper will be split into:

- ecological risk assessment (section 4)
- human risk assessment (section 5)
- synthesis of the discussion paper (section 6).

3.2.Current risk assessment approaches

3.2.1. Introduction

In current practices, risk assessment is generally defined as the procedure of assessing exposure and effects of chemicals (hazardous properties) in order to evaluate the risk for a defined biological target. While individual human beings are the target for human risk assessment, structure and functioning of ecosystems are the targets of ecological risk assessment. The term "environmental risk assessment" is often used when human beings are considered as organisms into the ecosystems (Calow, 1998). The methodologies used for human risk assessment and ecological risk assessment were developed separately in response to particular incidents. Nonetheless, for effect assessment, the strategy of using laboratory based tests on selected organisms as surrogates for species of concern is comparable although following opposite extrapolation mechanisms: extrapolation from several species to one in the case of human health versus extrapolation from few species to thousands or even millions of species (and to the complexity of biological communities and ecosystems) in the case of the environmental assessment. Both human risk assessment and ecotoxicological risk assessment methodologies have remained fundamentally unchanged for a long time although in both domains there has been a progressive addition of further tests.

Due to the introduction of new legislation such as REACH, the continuously increasing sensitivity of analytical systems to determine chemicals (both natural and synthetic) in ambient air, food, drinking water and natural water bodies, requests for assessing potential risks due to human and environmental exposures are increasingly demanded. This increasing demand may result in the need for major investments into hazard assessment and may overwhelm the regulatory bodies tasked with risk assessment. Moreover, advances in fundamental biological research identify potential new biomarkers of effects in a variety of biological systems and the relevance of these new developments for risk assessment also needs to be assessed. This discussion paper gives an overview on the wide variety of new developments in fundamental and applied research which may have relevance to health and environmental risk assessment procedures.

3.2.2. Meeting the needs of stakeholders

Although the purpose of this analysis is to identify future methodologies to advance the process of risk assessment, consideration of the use of risk assessment findings is necessary. Because risk assessments have primarily a practical purpose, it is crucial that the public trusts the process and that the findings from risk assessments and their implications are understood and provide a sound basis for action where appropriate.

Thus there are two important aspects:

- Transparency and trust in the process. It is important to make both the risk assessment process and its utilisation as explicit as practicable. This involves the sources of data considered, how the data for the risk assessment is selected and used and the uncertainties involved in the assessment. Risk assessment inevitably involves the need for scientific judgement. The less the data is accessible, the greater the need for such judgement. As a consequence, the process does and will always require a high level of expertise from the risk assessors. It is important to ensure that the risk assessors do not prejudge the data or have particular prejudices that would cloud their objective assessment of the data available to them. However, this must not be confused with complete independence, since this would limit the essential expertise available. The SCENIHR has developed a transparent procedure for showing how data is found, selected and used (the weighting of evidence) and how the uncertainties in the process are expressed. As new methodologies are introduced, it is important that their utilisation is incorporated into this weighting of evidence framework.
- Usefulness for risk management. A good risk assessment must ensure that the parameters considered are relevant, the findings are clear, properly disseminated and provide a sound basis for actions, where needed. It is important that the stakeholders, in particular the risk managers are involved with the risk assessment process, without distorting its scientific objectivity. The risk assessment needs to be couched in terms that are clear and provide a valued basis for actions. The risk assessment paradigm needs to take into account ways in which a risk can be helpfully contextualised:
  - *Against an agreed acceptable risk benchmark*: At present in Europe there is no definition of acceptable risk. Instead, it is often based solely on the application of very conservative, non-scientifically derived default factors. This is an issue that requires a dialogue among all stakeholders since its implications are much more far reaching than the domain of science!
  - *By comparison with other relevant risks*: This requires an available validated data base of risk assessments so that the most appropriate ones can be used for comparison purposes.
  - *Using a risk benefit/cost benefit framework*: Some of the European societies are considerably more risk averse than it is generally the case in the USA and many other countries. A presentation of risks devoid of any consideration of either the cost of risk reduction or of the benefits serves to reinforce risk aversion among politicians and the public. Cost-benefit analysis is one way of seeking to balance the benefits and costs of using chemicals and other stressors with hazardous substances.

Progress in the establishment of a transparent framework for cost/benefit analysis has been slow. This can in part be attributed to a lack of common understanding of the principles, practices, roles, techniques and terminology of risk assessors and economists. Interpretation of the same terms often differs between the two disciplines. For example, whilst risk assessors might consider population estimates to be the specific risk from a particular chemical to members of the public, economists might typically interpret such an estimate as signifying the population disease burden. The role of the risk assessors is to provide risk managers with scientifically defensible estimates of actual population risks, along with the variability and uncertainty associated with the risk. Often, risk assessments introduce very conservative default values to compensate for data gaps and other uncertainties. In this respect, they stray beyond the confines of science and enter into policy.

In order to facilitate the evaluation of risks and benefits alongside one another, assessments will need to be defined in terms that are compatible with the expression of benefits, e.g. economic terms.This is likely to include quality of life characterisation. This

was the subject of the recent discussion paper on Improvements in Risk Assessment (in preparation).

Effective stakeholder dialogue is essential in the development and implementation of the new paradigm for risk assessment. A particular issue is to ensure that the regulatory framework parallels the changes in the establishment of the roles of the new methodologies. If this is not the case it is probable that progress will be seriously limited.

There is a misleading and scientifically unjustified trend to classifying chemicals solely according to their hazardous properties. As the new methodologies are developed a dialogue with stakeholders should take place to enable a more appropriate classification system and to inform stakeholders of the risks. This is likely to be based on an improved understanding of modes of action.

In summary, although the primary purpose of this discussion paper is to identify the methodologies that can be used as the core of the future risk assessment paradigm, dialogue needs to take place with stakeholders regarding various factors that could have an important influence this development.

3.2.3. Meeting the need for flexibility and transparency

Many regulatory instruments define current risk assessment procedures.. Although this has advantages, it tends to reinforce a check list approach to risk assessment as opposed to an intelligent one and hampers the introduction of new methods. For example, in a number of domains it reinforces the application of standard default (uncertainty) factors.

As new methods are developed their role in risk assessment needs to be identified. They should not just be considered simply as further tests that should be conducted. The move to a new risk assessment paradigm focussed on an 'intelligent' approach will require high transparency both in the data generation and in its analysis for risk assessment purposes. This will put a high emphasis on how the data is selected and weighed. This is the subject of a memorandum of the SCENIHR (2012).

The development of the databases, in silico techniques, and understanding of modes of action is likely to enable a new scientifically sound approach to stressor classification. The current trend to a hazard based classification makes little scientific sense.

3.2.4. Meeting the needs of vulnerable populations

A major challenge in risk assessment is the protection of vulnerable populations, considering vulnerability as the combination of higher susceptibility (i.e. the presence of biological intrinsic factors affecting the response to a chemical), higher levels of exposure and additional factors that include social and cultural parameters (e.g. socio-economic status and location of residence but also risk awareness and risk education of each member of the popoulation) that can contribute to an increased health risk.

The exposure shows large variations as a function of life stage, due to changing physiology but also due to different lifestyles resulting in different behaviour. Exposure in early life may produce epigenetic changes that may not result in a risk but late life exposure to the same or a different compound may result in an adverse effect.

The evaluation of exposure to chemicals and the related health risk requires population-specific information that may vary significantly, depending on geography and cultural practices. In addition, exposure scenarios and response factors may vary for different populations based on age and life-stage changes in behaviour and physiology, which can determine critical windows of susceptibility. Although experiencing the same level of external exposure, some individuals can be more susceptible due to developmental stage or age, pathological status, or to genetic features affecting any individual's ability to withstand harm from a variety of chemical exposures. The internal dose, which determines the toxicological outcome, can be affected by the genetic polymorphism and

differential expression of active transporters or enzymes involved in the toxicokinetics of a given chemical.

The ability to identify vulnerable populations is increased by the knowledge of the mechanism of action of chemicals, allowing to consider the impact of factors such as age, genetics, environment, exposure, pathophysiological conditions or combinations of these and other factors on risk assessment protecting the overall populations, including vulnerable groups.

3.2.5. Expression of risks and benefits

It is anticipated that risks and benefits will be considered increasingly together. This will require the expression of risks and benefits in a common language and may have implications for hazard characterisation, assessment and expression. It may include estimation of probabilities and expression of conclusions in terms of composite indicators such as Quality-adjusted life years (QUALYs) and Disability-adjusted life years (DALYs). There will also be a need for a clear statement of the nature, extent and implications of any uncertainties.

3.2.6. Meeting the training needs

To ensure good progress towards the new paradigm for risk assessment recruitment, training and opportunities to gain relevant experience are essential. This will entail substantial changes in the requisite skills base. It is also necessary to recognise that the increased reliance on non-animal tests for both hazard and exposure assessment will, initially at least, require a much greater emphasis on the use of judgement by risk assessors. Consequently very experienced risk assessors will be needed. It appears very unlikely that the current availability of risk assessors will be sufficient to meet these demands. While training is needed in a wide range of competences, priority needs to be given to developing experts in exposure assessment since this should be the key factor in the future for risk assessments. Several levels of training for both exposure assessment and hazard characterisation may be recognised, namely:

- Basic knowledge/expertise. This requires a general understanding of the overall risk assessment process and how it is changing over time. Such knowledge/expertise is needed as the start of the training of risk assessors, and as a minimal requirement for data generators, risk managers and other stakeholders in a range of risk assessments.
- Advanced knowledge/expertise. This requires sufficient knowledge/understanding to be able to conduct a relatively straightforward risk assessment. At this level individuals should also have an in depth knowledge of either hazard characterisation or exposure assessment and the ability to work with experts in the other area.
- Outstanding knowledge/expertise. Such individuals would normally have extensive experience in personal research in a relevant area of risk assessment, an ability to work across the relevant disciplines and to tackle complex risk assessment problems. Individuals at this level would be expected to be able to contribute to the future development of risk assessments and to be able to act as mentors to individuals at lower levels.

3.2.7. Ecological risk assessment

General description

Currently used approaches for ecological risk assessment are based on the comparison between an indicator of exposure (e.g. Predicted Environmental Concentration or PEC) and an indicator of effect (e.g. a Predicted No Effect Concentration or PNEC) or an

ecotoxicological end point (e.g. a No Observed Effect Concentration (NOEC) or an EC/LC_{50}). Risk estimation is simply calculated as the ratio between these indicators (e.g. PEC/PNEC or TER: Toxicity Exposure Ratio).

The procedures for ecological risk assessment according to the requirements of European chemical regulations are described in detail in some official documents such as the Technical Guidance Document (TGD) on risk assessment (EC, 2003) and, for plant protection products (PPPs), in the Annex VI (Uniform Principles) of the Directive 91/414 (EC, 1991). Even if the details of the procedures are different, the conceptual approaches are similar.

A PEC for each environmental compartment (water, sediment, air, soil, biota) is generally estimated using multimedia modelling approaches applied to standardized environmental scenarios such as the local, regional and continental scenarios proposed by the TGD or the European agricultural scenarios proposed by the FOCUS group for PPPs (FOCUS, 2001a and 2001b). The first approach determines a long term static concentration being reached at the moment the emission starts, whilst the second approach gives an estimation of the dynamic concentration with respect to time and is therefore a higher tier exposure estimation. Experimental monitoring data may be used if available and suitable.

Also a PNEC must be calculated for each environmental compartment, using available ecotoxicological data. The traditional procedure is based on a relatively reduced data set corrected by application factors (AF) related to the degree of uncertainty and the amount of information available. If suitable information is available, a PNEC may be also derived using the Species Sensitivity Distribution (SSD) approach or higher tier data (mesocosm or field data) that improve the ecological realism of the assessment. In some cases, if toxicological information is lacking, approximated extrapolation approaches may be applied (e.g. equilibrium partitioning method for soil and sediments).

All currently used approaches refer to a generic European environment and do not explicitly consider the characteristics of potentially exposed biological communities and ecosystems.

Pros and cons of the current approaches

Current approaches for ecological risk assessment are relatively simple and may be applied successfully even if the available information is relatively scarce. The procedures are described in detail in official documents, are enough transparent and allow producing results with a good comparative value among chemicals. In case of high uncertainty, conservative worst case assumptions are applied, so the approaches are likely to be enough protective (sometimes overprotective) for ecosystems.

The major disadvantage of most approaches is the complete lack of environmental realism. The complexity of biological communities and ecosystems does not correspond to the simplicity of the approach taken. Therefore, the extrapolation of the results to really occurring natural conditions is highly problematic. Some of the major issues that may be the origin of high uncertainty are listed below.

- Exposure is calculated in a static (steady-state) scenario with generic environmental characteristics (compartments size, temperature, organic carbon, etc.) and does not allow evaluating the complexity of spatially and temporally varying environmental scenarios and discharges.
- Models used to predict exposure concentrations are incapable to handle numerous classes of chemicals such as apolar i.e. lipophilic substances, polar and dissociating substances as well as soluble and or insoluble nanomaterials.
- For most chemical, physico-chemical and half-life data needed to run the model are scarce (even if increasing) and not validated.
- The complex interactions among the different species in a biological community and the indirect ecological effects in the ecosystem cannot be evaluated from laboratory

toxicological data on a few indicator species. Even approaches based on larger data sets (e.g. SSD) consider the different species independently.

- The use of standard scenarios does not consider the characteristics of different ecosystems and their vulnerability to chemical stress factors. Therefore, current approaches cannot be applied for site-specific risk assessment. Site-specific approaches would be very useful for management purposes, as well as for the requirements of some European regulations (e.g. the Water Framework Directive, WFD).
- The interactions between the combined effects of toxic chemicals and other stress factors (e.g. temperature, oxygen depletion in water, water shortage in soil, etc.) or, more in general, their dependence upon environmental factors is largely unknown. Some effective approaches such as the Biotic Ligand Model (BLM), are of limited applicability (e.g. metals).
- The effects determined by highly variable exposure, such as intermittent peaks for pesticides, are not considered. The use of a time weighted average (TWA) is only a rough approximation.
- The use of higher tier effect assessment tools (mesocosms, field and semi-field data) substantially increases the ecological realism of the results. However, the application of this approach for regulatory purposes still presents some drawbacks (needs for methodological standardisation, transparent procedures for extrapolation, etc.).
- If they have been shown to be realistic, robust, and making correct predictions, ecological models can be used to overcome most of the current limitations of ecological risk assessment. They address the population or ecosystem level and they can, if designed for this purpose, extrapolate to new environments and conditions. They have been used in other fields to support EU regulations (wildlife epidemiology) but for risk assessment of chemicals, they are in their infancy.

This list of the drawbacks of current procedures for ecological risk assessment is far from exhaustive. However, it may provide a preliminary idea of the difficulties of using standard approaches to understand the actual consequences of e.g. the release of a specific substance onecosystem health.

3.2.8. Human risk assessment

General Description

Human risk assessment consists of 4 steps; Exposure assessment, hazard assessment, dose-response extrapolation, and species-extrapolation. The approach to human risk assessment used so far has been hazard-driven with a strong reliance on the use of laboratory animals as surrogates for humans.

The selection of rats and mice for this purpose was based primarily on animal husbandry considerations (e.g. ease of breeding, housing and maintenance) rather than scientific evidence that such rodents responded to chemical exposure in a very similar manner to humans. Other animal species such as dogs are only used in hazard assessment and determination of dose-response relationships for chemicals with intended human exposures (e.g. food additives, pharmaceuticals) or for chemicals with specific applications (e.g. plant protection products). For specific cases such as pharmaceuticals, non-human primates can also be used. In these cases, it is hoped that the use of an additional animal species (selected based on the expected similarity of toxicokinetics of the specific chemical in the experimental animal to that in humans) will reduce the uncertainty in extrapolation of effects in animals to humans. Histopathology is the accepted determinant for effects assessment along with changes in organ and body weight and some selected biochemical and haematological parameters.

Over time these tests have been increasingly standardised by the introduction of good laboratory practice and ICH or OECD test guidelines. Some *in vitro* tests, in particular for genotoxicity and topical effects have been added. Many of the tests in current use are written into legislative requirements for the approval of various types of products. To reduce the number of animals used and to enhance the likelihood of identifying an adverse effect, it has been common practice to dose animals at much higher exposure levels than humans would ever be likely to be exposed to under normal circumstances.

To address uncertainties due to the need for extrapolation, when using data obtained in rats and mice to characterise effects that may occur in humans, conservative standard default values (also called assessment factors, uncertainty factors or default factors) have come into common use (see Fig. 1). (EFSA 2011). The current approach uses default safety factors of 10 to account for species extrapolation between responses observed in experimental animals and those potentially expected in humans. An additional factor of 10 is used to cover inter-individual differences in response over the human population. This factor is also considered sufficient to cover potentially vulnerable subgroups, but in some specific cases the application of additional safety factors may be considered on the basis of experimental data, to allow for particularly vulnerable population groups. In case of an insufficient database, or of factors which may modify the responses, the default factors can be adjusted. For a very limited number of chemicals or groups of chemicals, mode of action studies have been used to characterise the soundness of the scientific basis for the extrapolation of data to man (e.g. organophosphorus pesticides and phthalate plasticisers).

The approach described above is used as a general framework and is usually performed for a single chemical or stressor. However, approaches to assess potential effects of combination of chemicals are presently developed (SCHER, SCENIHR, SCCS 2011).

In the development and validation of new methods it is essential to define the criteria for acceptance (often termed the gold standard). For example, in developing new methods for the assessment of risks to man, is reliable data in man the only gold standard or is good quality data obtained in laboratory animals a suitable alternative? The answer to this question inevitably influences priorities for development and use of databases. This answer is also vital in the selection of test systems, for example to ascertain the importance of using cells derived from man in developing *in vitro* systems. Cell culture systems and other biological models used in this context need to be stable and retain the human phenotype over prolonged periods of time. For the purposes of toxicokinetics, human PBPK models can be considered to be the gold standard but they need to be evaluated properly.

Exposure

The weakest point in many risk assessments is the characterisation of exposure, (external, internal dose). Consequently many risk assessments are qualitative or at most semi-quantitative.

Generally, exposure assessment relies on measured data where the chemical under consideration is determined in ambient media and the concentration in the ambient media can be transformed to predict human internal doses. Alternatively, the chemical of interest is directly determined in human populations by biomonitoring using biomarkers. Sufficient exposure data based on biomonitoring (biomarkers) are only available on chemicals of specific interest (e.g. phthalate esters, bisphenol A, some flame retardants) or with a high potential for toxicity (such as selected heavy metals, polychlorinated dioxins and biphenyls). In many cases, biomonitoring shows that the actual human exposures assessed by this direct method are much lower than the exposure assessment based on indirect methods, emphasizing the conservatism in exposure assessment. Since many of the factors determining actual human exposures may be highly variable within the population, conservative assessments of exposure are usually applied. Due to the time consuming and costly monitoring using measurements, reliable exposure data do not exist for many chemicals and exposures are often predicted based on models

integrating physico-chemical characteristics of the chemicals, occurrence or release data, and assumptions of human behaviour.

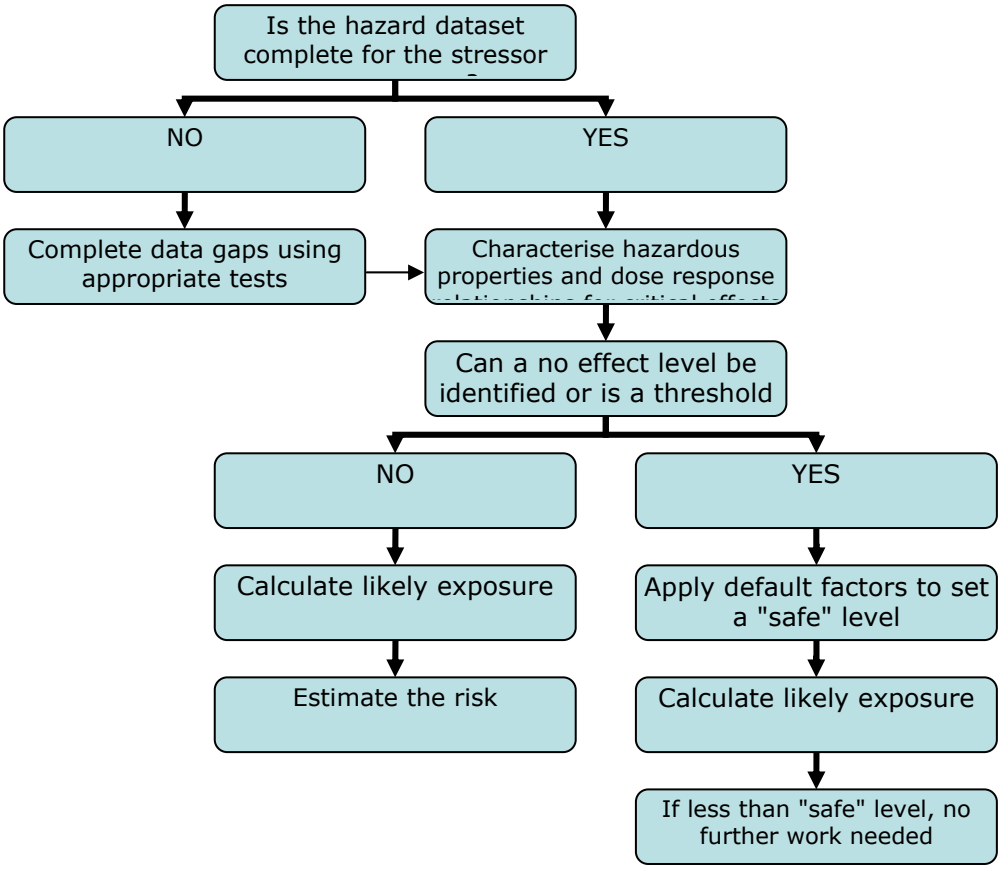
The quantification of exposure, both in individuals and in populations, is a prerequisite for the quantification of risk. Reliable data on exposure are needed to assess the probability of adverse effects of the stressor and to recognize specific risk factors such as occupation, life style, and social status. The dimensions of exposure include intensity, frequency, route, and duration; in addition, the nature, size, and makeup of the exposed population should be characterized. The assessment of exposure is a difficult and complex task. Typically, estimations and field measurements are required. The estimation of human exposure to a particular stressor involves an initial estimation of the possibilities for exposure. A good inventory of sources may provide important information on critical pathways of exposure, populations at particular risk associated to higher exposure, and the levels of exposure.

In many cases, the duration and level of exposure, especially after chronic contact, may only be estimated from ambient levels of the stressor in the environment, in food or in other media, and estimations may thus be crude. The external dose could represent a worst case, but only for those chemicals not bioaccumulating after repeated exposure. Owing to the large numbers of potentially exposed persons, only in special situations (e.g., occupational exposure, specific public interest) a more precise assessment of exposure will be available.

A further concern is that most risk assessment involve a specific use of a chemical and do not take into account other sources of exposure to the same chemicals or to other chemicals (mixtures) with similar modes of action. To ensure that these are taken into account consideration at the outset should be on the life cycle of the chemical, including potential for misuse and options for end of life fate.

Figure 1: Current general framework for human health risk assessment of chemicals[3]

[3] This is a general framework, and a number of exceptions exist, e.g. flavourings, which because low levels of exposure are involved, TTC is often used.



Hazard

Data on the effects of even widely used chemicals in man is typically very limited and difficult to access. It is clearly unethical to conduct direct studies aimed at deliberately provoking a marked toxic reaction in man. It is noted however that some carefully controlled studies are regularly conducted to assess the dermal irritancy of some personal care and other products. In the case of medicinal agents for human use, thresholds for adverse reactions are determined. In addition there is much data largely unpublished on patients treated in poison reference centres and on effects of workplace exposure to various chemicals and other stressors. Commonly this data is unavailable for risk assessment purposes.

The available hazard assessment techniques mainly rely on toxicity studies carried out in experimental animals; only the endpoints genotoxicity, eye and skin corrosivity and phototoxicity can at present be covered by validated *in vitro* tests. The *in vitro* genotoxicity tests were developed based on the presumed major mechanisms for cancer induction (i.e. genotoxicity). However, positive *in vitro* genotoxicity is usually not accepted as such in a regulatory context and *in vivo* confirmation is asked for. Animal toxicity studies have usually been conducted using high exposure doses to ensure that the hazardous properties of the chemical are identified, although using a limited number of animals. Estimates of effects have been largely dependent on the identification of pathological and histopathological changes in conjunction with body and organ weight changes. Only rarely does an assessment involve an evaluation of the modes of toxic action of a chemical.

While the animal tests provide detailed information on the toxicity profile of a certain chemical regarding all endpoints considered relevant, a large number of test procedures have to be performed to complete hazard characterization. The testing is time consuming, costly and involves a large number of experimental animals.

On the other hand, despite considerable efforts to develop *in vitro* and other alternative procedures (e.g. *in silico*), such methods are still in development and, at best, may be used to elaborate mechanisms of toxicity for integration into the risk assessment process. Just determining cytotoxicity in a cell culture gives only very limited information on the types and severity of effects to be expected from a stressor in an intact organ, since most of the test systems use cultured cells derived from a specific cell type in the organ of interest; interactions on a tissue level, which may be major contributors to the development of a toxic response can not be assessed. Limited knowledge on the detailed modes of action for organ damage and development of pathologies exists and the building of such knowledge is essential to develop more sophisticated *in vitro* systems which better mimic the *in vivo* situation. In addition, concentration-time curves for a stressor in an *in vitro* system may be widely different from those of the agent in a tissue of an intact organism, specifically after repeated dose applications as needed for risk assessment. Toxicokinetics cannot be studied in *in vitro* systems.

Extrapolation

The major drawbacks of the present risk assessment approach are the need for extrapolation for effect incidences in a limited number of genetically defined rodents to a large number of genetically inhomogenous humans. In addition, the present approach, due to the many uncertainties in the exposure assessment and the extrapolation needs is highly conservative in its basic assumptions and usually relies on worst-case scenarios in exposure assessment and effects assessment. The current process involves extrapolation from effect incidence at high doses to expected incidences of effects at the usually much lower actual human exposures. In many cases for non-genotoxic agents simple safety factors and the use of points of departure (e.g. NOAEL or benchmark doses) are used to perform extrapolations and specific adjustment to the default factors are only included in the assessment, when data are available and adequate justification can be performed.

The standardisation of the methodology has had the advantage of providing clarity for data providers, risk assessors and risk managers on what is required in the way of testing and the resource implications of this, and has benefited the development of structure activity data bases. In addition, the standardized toxicity tests provide points of departure (NOAELs, BMDs, TD50) as basis for the extrapolation of the results to humans. The approach is flexible due to the possibility to use different factors for deriving tolerable exposures to account for specific circumstances. Regarding cancer risk assessment, the present approach generates dose-tumor incidence data to be used to estimate and compare risks for setting priorities. Moreover, there is considerable experience in the application of this process to a variety of chemicals and exposure situations and the basic conservative safety factors usually result in sufficient protection of humans.

This standardisation of tests has probably led to too much reliance being placed on findings in rats and mice and as a consequence may have inhibited innovation in risk assessment procedures.

It is clear that the present risk assessment process also requires considerable experience in toxicology and a detailed understanding of procedures for toxicity testing, toxicokinetics, mode-of-action, and analytical and modelling systems to correctly assess the relevance and potential flaws both in toxicity studies and in exposure assessment. This will be even more so in the future.

3.2.9. The impact of REACH

The overall exposure of an individual to industrial chemicals can be summarised as the combination of the exposure from consumer products and the indirect exposure from the environment (ideally including food, water, indoor and outdoor air). REACH generates direct information on all consumer products defined as substances or mixtures in which

chemicals are incorporated and on the expected release including the waste/recycling phase. REACH is also generating information on the expected environmental releases of each substance along its life cycle. For workers, the additional workplace exposure should be considered, and specific exposure estimates are also provided in the chemical safety assessments.

The REACH exposure estimations cannot be just accumulated to obtain the overall exposure. First, because the information is generic and cannot be associated to specific citizens or ecosystems; and second because most exposure estimations are based on models and assumptions designed for covering "worst case" conditions, while the challenge is to estimate the "realistic" combined exposure. Nevertheless, the exposure conditions of a citizen or ecosystem can be presented as a combination of "exposure layers", defined by a set of parameters, e.g. dermal exposure from consumer products; oral exposure from food, etc. Each layer can be subdivided into other layers, e.g. depending on the type of products (cosmetics, furniture, cloth, toys, etc.), location (home, workplace, education centres, recreational centres, vehicles, etc), etc., allowing the consideration of additional factors such as age, gender, geographical location, etc. The "exposure layers" for ecosystems are linked to the geographical scale and can be defined using generic (e.g. local, regional, continental) and/or specific (e.g. lakes, rivers, river basins, coastal areas, etc.) descriptors. There are interconnections among the layers, e.g. the layer covering the exposure from food is associated to the environmental layers of the area in which the food is produced; the dermal home exposure and the indoor air home exposures are associated to the furniture, construction materials and domestic consumer products, and the local environmental releases due to municipal wastewater and urban waste management are related to the presence of chemicals in consumers products.

The challenge is to develop a new risk assessment paradigm, based on the integration of thousands of exposure layers, at the level at which the relevant chemicals or type of chemicals could be identified based on the use descriptors and use conditions described under REACH. Each layer should be defined up to a level allowing the characterisation of exposure patterns, e.g. relevant population group (e.g. by age, gender, activity, location, etc.) or ecosystem type (e.g. by ecotype, ecoregion, ecoservices and ecovalues), and the integration of other information sources (e.g. societal/cultural characteristics, population distribution, consumption behaviour).

Obviously, no deterministic quantitative estimations (e.g. for each European citizen or each ecosystem) can be afforded based on the current information. However, probabilistic distributions can be used to identify the likelihood for exposure to specific chemicals or groups of chemicals, and in some cases to produce probabilistic distributions.

Regarding the hazard characterisation, the information compiled for individual substances under REACH can be used for developing the hazard profiles of the substances relevant for each layer, in terms of combined effects (similar or dissimilar mode of action); in addition the information would allow setting commonalities and differences between the different chemicals that can be used as alternatives for a particular use; moving from chemical-by-chemical profiles to probabilistic descriptions of the "hazard profile" of the combined group of chemicals by use (for consumer's risk assessments) and by process (for environmental risk assessments). The notifications under the CLP Regulation also contribute to the assessment of the real hazard profile of the substances marketed in Europe, including impurities.

The next step is to combine the qualitative likelihoods and the probabilistic outcomes into exposure estimations, based on the EE related to each use; and then, to integrate the exposure and hazard profiles into "screening risk characterisation profiles" presenting the relative relevance of each exposure layer, and of the chemicals within the layer, in terms of the estimation of combined effects.

At this level, it should be possible to conduct a set of partial validations and calibrations based on complementary sources of information, e.g. the outcome of the REACH evaluation projects, monitoring data in humans, food, air, water, biota, etc., epidemiological studies, estimations for individual substances from comprehensive higher tier assessments, etc.

The re-calibrated model could then be used as a tool for priority settings, e.g. to identify the population groups and ecosystems with the highest potential combined risks, and even more important, the chemical combinations and exposure conditions leading to these highest risks. Confirmation programmes and/or risk management measures could be implemented.

The approach requires the integration of billions of individual data from different origins, mostly from aggregated sources (e.g. ECHA, EFSA, EEA, Eurostat, national statistics, risk assessment for priority substances, etc.), into workable assumptions for screening assessment. The aim should include the integration of thousands of exposure layers, weighting their relevance for particular human and ecosystem subgroups, as source for exposure assessment, and a new risk assessment paradigm based on realistic estimations of the risk in which the true variability is not hidden by worst case or averaged approaches and is the integral part of the risk communication.

The development should improve the possibilities for partial validations and include re-calibration tools; and consider that the amount of "REACH information" will be continuously growing in the future, and is expected to cover over 30.000 substances. However, this information should be openly available and its quality should be internally and externally validated through expert assessments.

An essential challenge when setting the new developments is to include the huge amount of complementary information already available or to be obtained in the short and medium term, related to the food, consumer protection and environmental arena.

As a medium to long-term goal, the information on combined chemical risk should feed the current and future European programs on health and environment including specific hazards (carcinogenicity, cardiovascular diseases, immunological responses, diabetes), and ecological values (such as biodiversity and ecosystem services). For human health issues the REACH-based exposure scenarios should provide estimations on which chemicals or chemical combinations could be related to subpopulation groups identified by age, gender, location, social/cultural behaviour, professional and recreational activities, etc. including temporal trends. For ecosystems, the scenarios should cover direct releases and indirect emissions associated to anthropogenic activities, such as municipal and industrial effluents or to waste management. The level of detail should allow the identification of possible associations among effects for each key health and environmental problem and past/present estimated exposure and chemical risks.

3.2.10. Conclusions on priorities for change and their rationale

It is very timely to review current approaches to risk assessment. There are major external pressures to change. It is also appropriate from a scientific viewpoint. Recent major advances in the understanding of biological processes, along with the increasing availability of rapid screening and data processing tools provides new opportunities and challenges.

A first priority must be to ensure that there are suitable accessible databases so that studies in animals are not repeated unnecessarily and that read across and (Q)SAR can be conducted with confidence.

A second priority is to improve current approaches to exposure assessment. Exposure information is often the weakest aspect in risk assessments. Moreover, in order to implement methods that limit the use of experimental animals, approaches such as thresholds of toxicological concern (TTC) are being introduced, and a reliable exposure assessment is essential to prioritise chemicals for hazard assessment. More efforts should

be given to the measurement of internal exposure: in this sense, if there is no possibility to have any systemic dose, the process could stop. Adler et al. (2011) indicate that kinetic info is crucial to understand 'internal exposure'. Usually when exposure is cited, the external exposure is implicitly the reference. It could be useful to introduce the concept of internal TTC and /or PBPK modelling.

A third priority is to develop improved understanding of modes of action of toxicologically important chemicals. This will provide an essential scientifically justified base for characterising threshold for adverse effects and identifying vulnerable population groups. It would also enable a sound basis for read across, a relevant framework for the grouping of chemicals and for the risk assessment of mixtures.

The development of *in vitro* testing strategies to determine relevant doses for toxicity testing for risk assessment purposes is another priority, but it is essential that the strategies foresee the integration of biokinetics data, identifying the actual level of cell exposure. The development of advanced *in vitro* models and testing strategies aimed to mode of action identification and early prediction of adverse effects could not be separated from the need of identifying actual *in vitro* marker of adversity. This priority has overlapping aspects with the already listed ones, but expands their area of applicability.

For ecological risk assessment, the main priority is developing tools capable to account for the complexity of natural ecosystems, both for exposure assessment (e.g. estimating bioavailability of chemicals in aquatic and terrestrial environments) and for effect assessment (e.g. considering the complex interactions among biotic and abiotic factors in a biological community).

4. ECOLOGICAL RISK ASSESSMENT

4.1. Objectives

The main objective for improving ecological risk assessment is to better protect ecosystems, in particular by:

- Extrapolating from lower levels (individuals) to higher levels (populations, communities) of hierarchical organisation;
- Extrapolating from the simple exposure patterns that are covered in laboratory tests to the complex exposure patterns that actually occur in ecosystems;
- Accounting for recovery processes; and
- Accounting for indirect ecological effects.

Current procedures required by chemical regulations only achieve a small part of these goals (Hommen et al., 2010).

Moreover, European regulatory framework for environmental protection is dealing with more than just chemicals and EU legislation is not limited to legal acts such as REACH, the pesticides directive and the biocides directive. Indeed, many important European directives are clearly oriented towards ecosystems (e.g. Water Framework Directive) and, therefore, require tools that can describe the complex interactions among ecological (biotic and abiotic) factors which may influence the way particular stressors affect local and regional ecosystems.

In this context, some specific objectives for an improved ecological risks assessment can be identified:

- To develop tools which go beyond the classical concept of PEC and are capable of describing more realistic exposure patterns for single chemicals and mixtures;
- To increase the ecological realism of effects assessment by improving the use of higher tier and field or semi-field data and/or introducing methods that can predict or assess ecological processes which are currently neglected in the RA procedures;
- To define good practice for ecological modelling and agree on scenarios and ecological endpoints so that models can be used to assess ecological risks rigorously for a wide range of species, regions, and environmental conditions.
- To improve the development of site-specific assessments taking into account the characteristics of exposed ecosystems;
- To account for the complexity of real ecosystems, considering the effects of biotic and abiotic interactions at community and ecosystem levels.

4.2. Exposure assessment

4.2.1. State of the science

In ecological risk assessment, exposure is generally assessed by evaluating the concentration of a specific chemical in the main environmental media (air, soil, water, sediment) and in biota. The latter is particularly important, as it is a food source for other organisms and, as such, could lead to possible secondary poisoning (EC, 2003). Exposure assessment is performed by evaluating the multimedia pathways of a chemical in order to establish the prevalent concentrations in the various compartments, either by direct measurements or by modelling approaches. Organism exposure is therefore derived

considering that the organism is living in or interacting with such media, *in lieu* of considering the internal concentrations as resulting from the exposure. This makes the assessment seemingly simple, but in reality potentially complicated if the many environments (and ecosystems) in which the huge variety of organisms live and interact are considered. For example, biomagnification (accumulation of a chemical through the food chain) can occur for certain types of molecules, and its intensity depends on the type and length of the food chain, which exhibits considerable variability in different ecosystems.

Environmental exposure can be assessed by means of experimental monitoring and/or predictive modelling. Both approaches have advantages and disadvantages and the choice for a particular approach needs to be based on a case by case evaluation.

Planning a monitoring programme raises a series of questions, such as: what parameters should be included? Where and when should they be measured? Moreover, without knowledge of the distribution and transport patterns of a chemical, monitoring data represent only single points in space and time, providing little opportunity for extrapolation to reconstruct a geographical distribution. Finally, environmental monitoring is an *a posteriori* approach, without any possibility for prevention.

Modelling is an *a priori* approach and represents the only possibility of generating information for preventive purposes and for estimating potential exposure before a new chemical is used. On the other hand, models cannot be calibrated and validated without experimental data. In order to avoid mistakes when applying predictive models, the proper model for the specific environmental situation must be carefully selected.

Taking into account the relative value and limitations of experimental and theoretical approaches, the best solution, where possible, could be a combined application of both methods, i.e. using models to ensure the proper planning of monitoring and better interpretation of experimental data and using monitoring data in order to avoid possible mistakes due to the improper use of models.

A number of general issues for monitoring and modeling approaches are listed below. Later on, monitoring and modeling approaches (with the respective issues) will be dealt with in separate sections.

4.2.2. Bioavailability

Organic chemicals

The assessment of and accounting for the bioavailability should play an important role in ecological risk assessment. However, until now information on bioavailability is not clearly included in the risk assessment process. Within the equilibrium partitioning theory, sorption to sediment and soil is included in the evaluation of the potential risk of sediment and soil pollution (Di Toro et al., 1991). Concentrations in the aqueous phase are then calculated from concentrations in soil or sediment and sorption coefficients. These sorption coefficients are often estimated from octanol-water partition coefficients (Kow) and QSARs for soil sorption (Karickhoff et al., 1979; Sabljic et al., 1995), thereby simplifying environmental sorption of organic contaminants as a single bulk partitioning process. These Kow based correlations do not take into account the possibility that sorption to soil or sediment can be substantially higher because of aging effects or sorption to phases with a high sorption affinity such as for example soot particles (Cornelissen et al., 2004; Gustafsson et al., 1997; Jonker and Koelmans, 2002; Naes et al., 1998; Sander and Pignatello, 2007). Bioavailability is not only an issue in soil or sediment, but may also play a role in the aquatic environment itself where binding to small particles (e.g. suspended solids) or dissolved organic matter such as humic acids may reduce the concentration that is available for uptake into organisms. It is well accepted that only the freely dissolved concentration is available for uptake via the aqueous phase (Hermens et al., 2007). Of course, small particles or food represent an

additional route of uptake, but it is often assumed that for relatively small organisms in the environment, the dissolved concentrations in the aqueous phase can explain the concentrations inside the organism at an equilibrium situation (Jager et al., 2003). Biomagnification via the food chain may occur and is often due to a non-equilibrium situation (Gobas et al., 1993). The freely dissolved concentration in the aqueous phase, but also in soil or sediment pore water, is therefore an important parameter for estimating the uptake and toxicity to "small" organisms. Also for modelling biomagnifications in the food chain, the information about the freely dissolved concentration is relevant because organisms at the bottom of the food chain (algae) are in equilibrium with the dissolved chemical in the aqueous phase.

The terminology around bioavailability is confusing. In ecotoxicology, the term bioavailability is related to, but not the same as, bioaccessibility. The concepts of bioaccessibility and bioavailability are explained in depth in a few review papers (see for example (Reichenberg and Mayer, 2006; Semple et al., 2004). As mentioned by Reichenberg and Mayer (Reichenberg and Mayer, 2006) bioaccessibility is related to "how much of a chemical is available for depletive processes", while bioavailability is related to the "direction and extent of diffusion and partitioning". Also the experimental methods to measure bioaccessibility and bioavailability are different. For measuring bioaccessibility, often depletive extractions are applied with extraction phases such as Tenax (Cornelissen et al., 2001), cyclodextrin (Reid et al., 2000), while for measuring bioavailability, partitioning based extractions are applied with a variety of sampling devices or phases including semi permeable membrane devices (Huckins et al., 1990), solid phase microextraction (SPME) fibers (Heringa et al., 2002; Mayer et al., 2000; Ter Laak et al., 2006), polyoxymethylene (Jonker and Koelmans, 2001) and polyethylene (Adams et al., 2007; Booij et al., 2002). Table 1 provides a comparison of both extraction techniques.

Both extraction techniques have their strengths and weaknesses. The depletive methods are suitable for estimation of the amount of a chemical in soil or sediment that can potentially be degraded, while the partitioning based methods are more suitable for evaluating the bioavailability in relation to bioaccumulation and toxicity. A brief summary of the approaches, including a brief guidance on how these approaches can be applied in ecological risk assessment, is given in the table below. For neutral organic contaminants, the development of the measurement techniques has reached a point where it can be applied in actual risk assessments in both the EU Water and Soil Framework Directives. Nice examples of the application of passive sampling methods in evaluation of soil and sediment contamination with PAHs are recently described in the literature (Hawthorne et al., 2008; Jonker et al., 2007).

Table 1: Comparison of extraction techniques for organic chemicals

Type of extraction	Depletive extractions	Partitioning based extractions
Parameters	Fraction fast desorbing.	Freely dissolved concentration - C (free).
Application in risk assessment	C soil-bioavailable = C soil total x Fraction fast desorbing Compare C soil bioavailable with quality criterium from toxicity test with "100 % bioavailability".	Compare C (free) in pore water or water with quality criterium from water only test.
Relevance	Estimation of mass that can be degraded.	Estimation of uptake and toxicity.

Another approach to assess/estimate bioavailability is the so-called body residue concept (Escher et al., 2011a; Hendriks et al., 2005; Landrum et al., 2003; McCarty et al., 2011; McCarty and Mackay, 1993). By using body residues instead of concentrations in the environment, bioavailability issues are avoided. In the absence of measured body residues, however, current risk assessment approaches apply a simplifying single bulk partitioning approach, based on K_{ow}. The latter representing the affinity of basic membrane lipids for non-polar organic contaminants. Whereas a hydrophobicity cut-off for membrane uptake is generally accepted for very hydrophobic compounds, this was recently challenged as an artificial phenomenon, resulting largely from measuring at non-equilibrated systems and reduced bioavailability (Jonker and van der Heijden, 2007). For polar and ionic compounds, uptake in exposed organisms may not solely depend on affinity for membrane lipids, but on other more complex permeation processes (Trapp, 2000) and additional binding phases such as proteins (Endo et al., 2011).

Bioavailability is not only an issue for *in vivo* testing; it is also important for *in vitro* testing. The outcome of *in vitro* tests may strongly depend on loss processes, but also on binding to proteins in the medium (Gülden et al., 2002; Heringa et al., 2004) as well as on the dosing procedure (Tanneberger et al., 2010). These phenomena lead to a large variability in effect concentrations from *in vitro* tests. More insight into the actual and available concentrations in these *in vitro* tests is essential in the extrapolation from *in vitro* to *in vivo* data (Gülden et al., 1994; Kramer et al., 2009; Schirmer, 2006). More insight into bioavailability is also essential in the application of physiologically based pharmacokinetic modelling (PBPK) to predict the *in vivo* kinetics and in bioaccumulation modelling (Escher et al., 2011b; Nichols et al., 2004; Nichols et al., 2006). Also, new dosing procedures in *in vitro* and *in vivo* testing (passive dosing techniques) (Kramer et al., 2010; Kwon et al., 2009; Mayer and Holmstrup, 2008) will lead to more robust data for effect concentrations. A recent overview of the bioavailability of xenobiotics in the soil environment is given by Katayama et al. (2010). In the paper a distinguishment is made between the bioavailable fraction of e.g. pesticide residues in soil and the bound residues, i.e. the fraction that is bound into the soil or sediment to an extent that it is not easily released from the matrix except by vigorous extraction techniques. Approaches are presented how the bioavailable fraction may be used in higher tier models.

Inorganic chemicals

Bioavailability is also a relevant issue for inorganic contaminants, particularly for metals. Metal bioavailabilty is a function of metal speciation and metal complexation (to organic and inorganic ligands) which determines the free ion activity (Allen and Hansen, 1996), and of interactions at the site of toxic action (Di Toro et al., 2001). The Biotic Ligand Model approach (BLM) (Di Toro et al., 2001; De Schamphelaere and Janssen, 2004) offers a means to account for both. The main assumption of the BLMs is that metal accumulation and/or toxicity results from the reaction of free metal ions with binding sites at the organism-water interface (either physiologically active sites, leading to a direct biological response, or transport sites, leading to metal transport into the cell followed by a subsequent, indirect biological response), which is represented as the formation of a metal-biotic ligand complex. The concentration of this metal-biotic ligand complex determines directly the magnitude of the toxic effect, independent of the physical-chemical water characteristics of the test medium.

Extensive research efforts have not only developed BLMs with various model organisms for predicting acute toxicity effects of metals (i.e. mainly mortality during short exposure periods) but also chronic BL models(i.e. not only effects on survival but also on growth and reproduction) (e.g. Heijerick et al., 2002; De Schamphelaere et al., 2004).

These (chronic) BLMs have been proved a powerful tool for assessing metal bioavailability and have been already applied successfully in EU risk assessment exercises reports (ECI, 2006; TNO/RIVM, 2006; Danish EPA, 2008).

To date, BL models have been developed for a number of metals (Cu, Zn, Ni) using a limited number of selected freshwater model test species. However, the developed models have been validated for other non-model species.

Clear needs for the bioavailability assessment of metals are the (further) development of BL models (or BL like) for the marine environment and for the evaluation of metal mixtures.

4.2.3. Monitoring

Current use

A huge amount of monitoring activities is carried out for various purposes from local to international level. However, the use of the resulting data is often limited to the respective programmes. For a generalized use of monitoring data there is a need for better harmonization of sampling, analysis and reporting procedures. Important issues in this context will be the accessibility, representativeness and accuracy of the data.

Large-scale monitoring programmes may be a good source of data, generally easily accessible. Moreover, there are also small surveys, generally planned for specific objectives, which could theoretically be used more widely than for the original purpose if they were easier to access. However, the use of these data is often difficult due to a lack of comparability. The data available should be harmonised at least to the extent that they follow a similar format and provide the necessary information to understand why a monitoring activity was planned and what the available data really represent.

Important pieces of information are the criteria used for the planning of the monitoring activity, such as:

- Criteria for the selection of chemicals to be monitored;
- Criteria for the selection of the environmental compartment;
- Criteria for the selection of the sampling sites and times.

These criteria may derive from sound scientific bases, such as a careful land use survey supported by modeling approaches. In these cases it would be possible to know in advance which chemicals are more likely to be detected, in which compartment(s) they will be more abundant, which is the meaning of different sampling sites (hot spots, background conditions, spatial and temporal variability etc.). The usefulness of these data for risk assessment may be very high.

Other kinds of criteria may be based on public concern (e.g. only water was sampled, close to drinking water supplies and only the "most toxic" chemicals were analysed), on practical reasons (e.g. availability of simple and cheap analytical methods) or on other non-scientific issues. In these cases, the usefulness of the data for risk assessment is poor or negligible.

Increasing spatial and temporal resolution

Given the practical and cost constraints related to measuring the environment, monitoring data are generally available at low temporal and spatial resolution. This means that they can seldom catch the variability in time and the relative spatial gradient of concentration change in a particular region. Sometimes, when the amount of spatial data is sufficient, geostatistical techniques can be used to reconstruct a spatial trend (e.g. in soil) but they are usually limited to static representations of contamination at a certain point in time.

Monitoring techniques generally provide average concentrations in environmental media (air, water, soil, sediment). Peak concentrations are generally obtained when the

measurement is performed at the point of discharge (air, water). While these data are important to estimate the order of magnitude of emissions, they generally do not allow catching the variations of concentrations in an environmental medium, because the chemical can undergo a series of transformations and transfers among media before reaching the point of measurement.

As monitoring data are used to gather a picture of contamination of a certain compartment or a certain environment or ecosystem and later used to "calibrate" or "validate" (or bench-mark) environmental fate models, their lack of details about the variability in time and space often gives a misrepresentation of the real phenomenon, which may show larger variation. This has been shown recently by Gasic et al (2009) who measured air concentration changes at short time intervals (6 h) of some PCBs in Zurich, showing that concentrations follow a daily pattern and that a better representation of the air compartment was needed in multimedia fate model to follow such changes. Gasic et al. (2009) suggested to implement some forcing functions to follow the planetary boundary layer (or mixing height) change during the daily cycle. Later, Morselli et al (2011) developed a multimedia fate model which implemented a dynamically changing air compartment which is better suited to account for such variation. However, the lack of measured concentration data set at shorter time resolution (1 h or less) for most of the organic air pollutants does not permit a full validation of the model in other environmental situations.

Collecting data for chemical mixtures and nanomaterials

Monitoring data of chemical mixtures are often very scarce for the main environmental media, apart for classes of chemicals (such as PCBs, PAH, PCDD/Fs), which are nowadays mostly measured in their individual components. The same situation is true and perhaps more serious for metabolites, which are rarely measured in environmental samples. This could be particularly important for biologically active chemicals such as pesticides (Verro et al. 2009) and pharmaceuticals (Deblonde et al., 2011). More details on the issue of mixtures in human and ecological risk assessment are described in depth in a recent Opinion of SCHER, SCCS, SCHENIR (2011).

Nanomaterials, especially the engineered nanoparticles (NPs), are a new source of environmentalcontamination. While their use is increasing, little is known about their use and release, fate and toxicity. Metal oxide nanoparticles (TiO_2, SiO_2, silver, iron) are among the most widely used types of engineered NPs. However, measurements in the environment are scarce, mostly due to the lack of methodology for the detection and characterisation of engineered nanoparticles in complex matrices, i.e. water, soil or food (Nowack and Bucheli 2007; Tiede et al., 2008).

Improving the quality of data

Additional information required to assess the usefulness of monitoring data are related to methodological details (for sampling and analysis) and to accuracy, precision and reliability. It follows that, for the use of monitoring data in ecological risk assessment, the availability of accurate and detailed metadata is even more important than the data themselves. A list of quality criteria for the use of monitoring data in risk assessment has been proposed by OECD (2000) and is detailed in the European TGD (EC 2003).

Develop new techniques

Some new sampling techniques for the environment have been implemented and used, such as artificial passive samplers, which can be used to record equilibrium concentrations in a certain phase such as: in air with shielded polyurethane foam (PUF) devices (Shoeib and Harner 2002), or other types of devices (Hayward et al, 2010); in water with Semi Permeable Membrane Devices (SPMDs) (Huckins et al, 1990, 1993) or Solid Phase Micro Extraction (*S*PME) (Heringa et al. 2002; Mayer et al. 2000; Ter Laak et

al. 2006). Similarly, some efforts were devoted to measure the equilibrium concentration in soil water using special devices derived from solid phase extraction cartridges (SPE). Artificial passive samplers have advantages over the "natural" passive samplers (such as leaves) in a way that they can be standardized and cleaned up before sampling in order to obtain comparable initial conditions. Passive samplers have the advantage of being capable of sampling a phase (e.g. air) for relatively long time (weeks, months) and therefore of capturing chemicals present at very low concentrations. However, their sampling rate depends on a number of environmental conditions (temperature, wind speed, etc.) and chemical specific physical-chemical properties, therefore they generally provide order of magnitude estimates. Current passive devices also have the disadvantage of generally providing average concentrations of the sampling period and missing the information on peak concentrations. Although clearly offering new possibilities for monitoring, more research on passive samplers is needed.

4.2.4. Multimedia fate models

State of the science

Multimedia fate models (MFMs) are nowadays among the most used tools to evaluate the fate of chemicals in the environment, due to their general predictive nature and the substantial ease of implementation. EFMs generally require data on chemical properties, environment conditions, and rates or quantities of chemical discharges and produce a picture of the environmental fate of a chemical, often in the form of a mass balance (Mackay and Mackay, 2007)

Many MFMs models have been developed in the past thirty years. Most are steady state models (in which chemical discharge is constant in time), such as the EQC and ChemCAN models (Mackay et al, 1996 a, b, c) or the European Union System for the Evaluation of Substances (EUSES) model (EC, 2004). Some were later developed as unsteady state or dynamic models (historically in terms of chemical discharge, now also in terms of environmental scenario changes). The steady state models are better suited to situations in which chemical emission does not significantly vary during a certain period of time (e.g. sewage treatment plant (STP) discharges. They are generally adopted for the simple mass balance equations obtained in such situations and because they do not oblige the user to provide a time-varying emission profile (at hour, day, week, month, year level). Dynamic models are better suited to handle episodic discharges, such as pulse discharges (e.g. pesticides) or mass movement of chemical caused by some environmental phenomenon (such as a runoff event triggered by rainfall).

Environmental fate models can be physical dispersion models (used to calculate concentrations in a phase (air, water, soil) in the proximity of a point source or partitioning or compartmental models, also known as Multimedia Fate Models (MFM). The latter can be built with different types of spatial resolution, presenting large compartments (regional) or smaller or site specific description (local), sometimes nested (a local model into a regional one), such as in the model EUSES (EC 2004). Compartments can be discretised vertically or horizontally: in the vertical discretisation each compartments can be made as one box or layered (e.g., soil, air, water, sediments) while horizontally compartments can be described with regionally averaged properties (organic carbon, texture, hydrological data etc) vs. distributed (typical/spatially explicit) properties (often supported by a geographic information system (GIS).

The European System for the Evaluation of Substances (EUSES) is the software implementation of the Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances (TGD). The EU risk assessment approach is generic in two ways: (i) it considers generalized hypothetical local and regional scenarios in which releases are assumed constant, at least during episodic events, and (ii) it assumes that transport and fate of chemicals follows in

a predictable way from physical and chemical substance properties. The EUSES model thus predicts space and time averaged concentrations of well-behaved chemical substances in non-existing hypothetical exposure situations. While this serves the valuable purpose of systematic treatment of substances in a fair and equitable way, this puts limitations to the reality of the risk assessment at the same time.

Limitations of EUSES

The major limitation of the EUSES exposure calculation is the low ability to relate predictions to the results of monitoring studies. Monitoring programs are usually designed to detect possible high-end risk situations near known emission sources, whereas by nature, the generic EUSES scenarios reflect different release situations, often more characteristic of background exposure. EUSES-predicted concentrations are valid only for when emissions are constant at an average level, and have been so for a considerably long period of time, so that exposure concentrations have come to steady state. In reality, this is seldom the case. Releases usually vary in space and time and often change quite rapidly, allowing less time than required for development of steady state. As a consequence, measurements cannot generally be used to test correctness of exposure predictions made with EUSES.

Another limitation of EUSES arises from its well-behaved-chemical assumption. Algorithms used were mostly derived for neutral, hydrophobic chemicals and work well for many not too hydrophilic substances, of which inter-media (air-water, water-sediment, soil-air) partitioning can be predicted from vapour pressure, water solubility and octanol-water partition coefficients of substances. In principle, EUSES can be used to predict exposure concentrations of other chemicals, provided that inter-media partition coefficients are put in directly. However, this possibility is not used often. Useful numeric values of inter-media partition coefficients are seldom available and EUSES is often used outside its domain of applicability.

Moreover, EUSES was designed with a number of hard coded environmental parameters which simulate a region with the characteristics of Holland. While this is reasonable for a generic European region, the conditions may not be adequate to simulate other regions in Europe. For this reason the EU FOCUS models, such as those applied to predict the fate of pesticide in ground and surface water (FOCUS, 2000, 2001) are applied within regionally developed scenarios. For example, in FOCUS Groundwater nine standard combinations of weather, soil and cropping data which collectively represent agriculture in the EU were assembled

In contrast to the wide-spread misconception that mass balance models can only be applied to hydrophobic chemicals, it should be noted that EUSES does not suffer from this limitation. It is true that many "fugacity models" are entirely Kow-driven in that they predict inter-media partitioning from hydrophobicity only. At first sight, this may seem to hold for EUSES, too. In reality, the limitation of most mass balance models (certainly that of EUSES) is only that chemical fate is driven by the tendency of chemicals to move toward thermodynamic equilibrium. For chemicals that are well-behaved in this sense, EUSES can be used, provided that inter-media partition coefficients are known or can be predicted (e.g. from Kow). EUSES has been used successfully for heavy metals, starting from empirically derived sediment-water and soil-water partition coefficients.

In order to use EUSES from physical-chemical substance properties only, algorithms must be available to predict inter-media partition coefficients. Such algorithms are indeed available only for the more hydrophobic chemicals, although EUSES is equipped with QSARs for soil-water sorption of many other, more hydrophylic chemicals, too. However, recent literature indicates (Franco et al., 2010) that a large fraction of the chemicals that are currently being registered under REACH are hydrophilic and/or ionize in water. In practise, this implies that, currently the applicability of EUSES is limited to those classes of (neutral) organic chemicals for which sorption QSARs are offered. Therefore, EUSES cannot be applied to very hydrophilic substances, such as detergents and ionizing

substances (e.g. acids with pKa>7 and bases with pKa<7). First of all, EUSES should not be applied to chemicals for which departure from thermodynamic equilibrium is not the main driver. This may apply to strong detergents; this is certainly the case for nanomaterials. As pointed out by Quik et al. (2011a, 2011b) colloidal aquatic suspensions of nanoparticles are inherently unstable and tend to aggregate or associate with other solids surfaces present. For nanoparticles, the solid phase is by far the most favourable state; drive towards it is not the departure from equilibrium (which generally is extremely large), kinetic limitations, which may result from presence of electrical charge. Solid-water partitioning of nanoparticles is controlled by processes that are currently not even modelled in EUSES.EUSES models one chemical at the time. It is not designed to account for the presence of other trace substances. In general, this is believed not limit use of EUSES to mixtures of substances, provided that the members of the mixture can be modelled individually. Difficulties of assessing the risk of mixtures arise and are dealt with at the stage of effects assessment.

The TGD excel sheet (EU TGD 2003 Risk Assessment Spreadsheet Model) is based on the same calculations and assumptions and may be an alternative of using EUSES. The tool offer environmental exposure estimation and the assessment of man exposed via the environment. The possibility for linking different excel sheets can be used for simultaneous exposure estimations for several chemicals, although with the limitations described above for individual chemicals. It should be noted that the local assessment would estimate the combined exposure assuming that all substances are releases to the same wastewater treatment plant.

Account for temporal and spatial variability

The complexity and variety of ecosystems in Europe implies the need of predicting the extent of concentration changes in time and space, to better characterize organism responses which may vary according to their life cycle and contribute to short and long-term effect on ecosystems, on a temporal scale different than for humans. Temporal scales are important when considering variations in chemical emissions (constant vs seasonal, pulse or event driven (i.e. runoff events)), variation in environmental scenario (temperature, precipitations, wind speed and direction), and prediction time scale, which can be shorter than monitoring time scale (i.e. hours, see Liess et al. 1999): daily concentration variations in air and exposure/uptake/release → need for measurements at shorter time scales.

Such temporal variability was shown for air concentrations of semi-volatile organic chemicals (SVOCs) which were measured in several field studies over daily (i.e., 24-hour) periods, with samples taken every 4 or 6 hours. The results had shown in some case, regular patterns: for example, the PCB concentrations measured (Lee et al. 1998) at a rural site in England were maximum during the day and minimum at night and their variation was ascribed to temperature-mediated air-surface exchange. Other studies showed maximum concentrations at night and minimum during the day and were related to factors such as the degradation reactions with OH radicals occurring during daylight hours (Mandalakis et al., 2003) or the diel variability of the planetary boundary layer (PBL) structure (Gasic et al., 2009). The importance of PBL dynamics and, more generally, of meteorology, in determining the concentrations of pollutants is well documented in the literature (Oke, 1987; Stull, 1988) but multimedia models generally ignore this aspect, with a few notable exceptions (Ma et al., 2003; Hansen et al., 2004; Sehili and Lammel, 2007); these integrated models are generally based on regional averages of environmental parameters and can be used to investigate the fate of chemicals at spatial scales greater than 1000 km^2. More recent efforts have recently shown the short-term fate of SVOCs at smaller scales, such as in Gasic et al. (2009) who used an existing multimedia mass balance model (modified using forcing functions for the air dynamics) to interpret the short-term variability observed in air concentrations of some PCB congeners in Zurich. More recently, Morselli et al. (2011) developed a multimedia fate model with two variable height air layers, hourly changing according to

the PBL variations. They showed that the amplitude of air concentration fluctuations (up to a factor of 5-30) modelled in concentrations could occurred in a very short time (few hours), in response to the rapid meteorological variations. Therefore they called for more frequent (hourly) measurements, since such rapid changes are rarely detected in air concentration measurements, especially with SVOCs, due to the general need of a longer sampling time to collect detectable amounts.

A better prediction of temporal changes could allow identifying situations and chemical uses (or environmental conditions) for which the standard "steady-state" approach (static emission and static environment) is inadequate to protect sensitive targets. One such example is a pulse emission, resulting either from a direct episodic discharge or from the action of sudden environmental events (rainfall or snow events, runoff from soils, sudden air boundary layer change, wind speed, etc.). Such variability needs in turn a more frequent monitoring of concentrations, e.g. at a time scale of hours. Sometimes these concentration variations could be quite large (more than an order of magnitude, albeit for shorter times) and would require modelling or evaluating organism exposure, uptake, release and toxicity (at acute or chronic levels) at shorter time scales. There is also a need to predict chemical concentrations at spatial scales different from those currently done, due to the variability of habitats, climates, emissions (use of chemical), scenario properties, which may range from site specific to regional or global. Also the discretisation of scale could hide important variation in properties, which could be fully described with a more detailed horizontal discretisation of space, therefore moving from large homogeneous regions, with standard or averaged properties to more spatial explicit model, also coupled with GIS. Also the vertical discretisation (e.g., with the adoption of layered compartments in air, water, soil, sediments) could help to better describe the extent of chemical concentration variations (e.g., variable height air compartments or layered soil models). This is shown by the generally better estimates for mobile (air, water,) than less mobile compartments (soil, sediments) and biota (up to 1-3 order of magnitude). Soil compartments in regional/global models are generally poorly predicted. As a consequence, there is a need to address uncertainty of the environmental (i.e. landscape) parameters: in a recent evaluation of EUSES platform using a representative set of compounds, uncertainty in environmental parameters typically contributed more to overall output uncertainty than uncertainty in substance parameters (Armitage et al. 2007).

Within the framework of the plant protection products directive of the EU a forum for the evaluation of pesticide fate models and their use (FOCUS) has been established with the intention to develop higher tier models for the estimation of the PEC. For the compartments soil, surface water and air the availability of suitable models has been inventoried and later on been made applicable to the estimation of time varying concentration of pesticides after application. The FOCUS Groundwater Working Group defined a set of 9 European scenarios for which available models have been parameterized. The available models are MACRO, PEARL, PELMO, PRZM. The calculated groundwater concentration at 1 m depth are used for the decision making process for PPP. In the FOCUS Surface Water Working Group a set of 10 EU scenarios have been defined including different types of surface waters, pond, stream and ditch. Some scenarios are suitable for drained land in European agriculture and some for typical run-off land. The different entry routes of pesticides, drift, drainage and run-off are successively combines as input to a surface water model describing the fate of the pesticide in surface water leading to time dependent concentrations as required for the assessment of aquatic organisms. The models used here are MACRO for drainage, PRZM for run-off and TOXSWA for fate in water. (FOCUS, 2001a and 2001b)

Enlarging chemical domain of applicability beyond the classical non-polar organic contaminants

Most of the monitoring and modelling tools have been developed for neutral and non-polar organic contaminants. Although sampling devices for more polar chemicals exist

and have been applied (Alvarez et al., 2004; Macleod et al., 2007; Vermeirssen et al., 2005) this field of research needs more attention. Most emerging contaminants are polar and ionized compounds including several pharmaceuticals, mycotoxins, veterinary antibiotics, biocides and surface active chemicals. It is expected that the focus within REACH (Franco et al., 2010), but also for example in the Biocide Directive, will shift to these more complex molecules. The simplifying bulk partitioning estimations based on octanol-water coefficients may lead, for these types of chemicals, to erroneous risk assessments. New ways of predicting sorption affinities are required (Bronner and Goss, 2011; Endo et al., 2011; Kipka and Di Toro, 2009). Although ionized and polar chemicals may, in general, show relatively low octanol-water distribution coefficients, positively charged compounds often have relatively high binding affinity to all kind of oppositely charged environmental surfaces (Brownawell et al., 1990; Carrasquillo et al., 2008; Richter et al., 2009; Sibley and Pedersen, 2008; Tolls, 2001). Soil/water partition coefficients (Kd) cannot be accurately predicted and one has to resort to measured (and of course site specific) Kd. The sorption of these ionized compounds to organic matter, but also to clay minerals, depends on salinity and pH (Mackay and Seremet, 2008; Sibley and Pedersen, 2008; Trapp et al., 2010). Also sorption of neutral and ionized surface active chemicals can be relatively strong (Droge et al., 2009; Higgins and Luty, 2007; Rico-Rico et al., 2010). More research into how to progress in modelling of these processes for ionized and polar chemicals is definitively needed (Goss and Schwarzenbach, 2001; Schwarzenbach et al., 2006). With these more specific chemical structures, also a change in focus is needed from bulk partitioning in organic matter to more specific phases in the environment, including ion-exchange processes on humic acids and clay minerals in soil and sediment. Furthermore, to better asses uptake processes and modes of toxicity in exposed biota and in toxicity studies for polar and ionic emerging compounds of concern, a change in focus is needed form bulk partitioning to membrane lipids to including binding to proteins (Debruyn and Gobas, 2007; Endo et al., 2011) and receptor sites in target tissues of exposed biota.

Besides the problem of polar/ionic chemicals, misleading results may be obtained when models (and equations) are applied outside a certain upper Kow value, for example for some highly hydrophobic chemicals, for which bioavailability could drop considerably.

Finally, predictive approaches applicable to nanoparticles/nanomaterials are not available at present. Considering the growing concern of these contaminants, the development of suitable models is an urgent need.

Increasing the ecological significance of scenarios

As outlined above, there is a need to improve the spatial and temporal variability of the scenarios adopted in modelling approaches to better reflect the variability of ecological conditions and environmental parameters in different territories. The FOCUS approach (FOCUS 2001a, 2001b), for which different environmental scenarios and parametrisations were adopted to account for variations in the fate of pesticides in groundwater and surface water, has addressed this issue partially by using a number of "typical" environmental scenarios. While this still represents a huge simplification of the real environmental conditions of the different areas, it is a major advance in the prediction of the fate of chemicals under different environmental conditions.

The variability of scenarios is best taken into account by using non-static models. Spatial variability is due to the different properties of the environment in different places and their time-related changes. Examples of this variability are the changes in organic carbon distribution in environmental media at different times or the variation of contaminant degradation velocities in different areas. In order to account for these temporal variations in scenarios, dynamic or non steady-state modelling approaches should be implemented. This is particularly true when population and community exposure is evaluated, since the presence of the organisms, their growth and their interactions depend on environmental conditions. However, models, especially those used for regulatory purposes, are generally

incapable to catch such interactions and to evaluate their importance. The current EUSES model for example, is based on a steady-state approach where time is invariant.

Overcoming the lack of understanding of processes or mechanisms

In terms of lack of understanding, a major issue is the integration of sub-models into complex models, especially when including or connecting a trophic chain (aquatic or terrestrial). This is shown by the general difficulty of defining the role of chemical contamination in ecological interactions (e.g. characterizing and defining the parameters of prey/predator relationships). The models currently in use, especially for regulatory purposes, do not include a dynamic vegetation compartment in the chemical mass balance in spite of the fact that the role of such a compartment in filtering or sequestering and metabolizing chemicals from air and soil is now clear (Nizzetto et al, 2006a, 2008a). Also the role of vegetation as secondary source of chemicals to air is not currently implemented. Litter fall (transport of leaves to soil) and its interaction with the detritus cycle, as well as the connection between carbon cycle and contaminant cycle, is now at its infancy (Moeckel et al, 2008, Nizzetto et al, 2010).

4.2.5. Bioaccumulation models

State of the science

While MFMs are used to predict the fate of chemicals in the main environmental compartments, they are generally incapable to accurately calculate concentrations in biota since they only provide estimates of bioconcentration. When more realistic concentrations are needed other approaches are required. Among those, bioaccumulation models (BMs) are predictive tools designed to evaluate the concentrations that a chemical could reach in an organism (aquatic or terrestrial) basing on the concentrations in environmental media (e.g., water, sediment, soil, air). The knowledge of the concentration reached in the organism or in certain tissue or organ is important to interpret the toxicological response of organisms. These levels generally depend on the interaction between the organism and the environmental media directly, but sometimes (when the chemical possesses certain physico-chemical characteristics and adequate metabolic half-life) a trophic enrichment can be observed. For the aquatic organisms three main phenomena can be cited: bioconcentration (process in which the chemical concentration in an aquatic organism exceeds that in water as a result of exposure to waterborne chemical, usually where the chemical is adsorbed only from water via the respiratory surfaces such as gills and/or the skin), biomagnification (process in which the chemical concentration in an aquatic organism exceeds that in the organism's diet, due to dietary absorption) and bioaccumulation (which sums up all possible routes of exposure) (Gobas, 2000). For terrestrial ecosystems similar concepts are adopted for terrestrial animals while plants are generally considered in terms of accumulation of chemical present in soil or in the air compartments. For the aquatic organisms, initial approaches were simple correlations (Kow based) mainly to predict bioconcentration in fish. The organism was considered as a single compartment. A full mass balance could be compiled, usually in steady state formulations. Then more complex models appeared and more processes, detailing uptake and clearance of chemicals, were introduced. Processes were gill ventilation, food uptake, egestion, and metabolism, as well as reproductive losses. Mechanistic explanations were found to describe the process of bioaccumulation, separating its two components, bioconcentration and biomagnification. Role of food vs. gill uptake was shown, especially for POPs (Persistent Organic Pollutants) important in quantifying the importance of the biomagnification processes in determining the uptake of POPs in the trophic chains. Bioavailability of chemicals in the environmental media (such as air, water, sediment, and soil) and in food was then investigated and it was

shown to influence the fraction available for uptake. The mechanism of uptake and release in aquatic air-breathing organism was later understood, showing that water respiring organisms were more efficient in clearing chemical than air respiring organisms. Therefore air-breathing warm-blooded animals, which require more food, were found of being subject to greater bioaccumulation and biomagnification from food (Mackay and Fraser, 2000). Other models were later developed for other aquatic organisms, such as benthic invertebrates, birds, up to marine mammals (Hendriks et al., 2001; De Laender et al., 2009). Some of the models were physiologically based toxicokinetic (PBPK) models similar to those developed for pharmacokinetic studies in toxicology.

These single species models were then sometimes integrated in more complex food web models, allowing the reconstruction of chemical accumulation through food chains or webs. The increase in complexity required a consequent increase in species-specific parameters (which are seldom available) forcing the models to be based on "generic" physiological parameters (such as egestion or metabolic rates) and relying on the available specific parameters (such as lipid fraction or ventilation rate). While most of the efforts were put in aquatic food webs, a consistent lack of knowledge exists for terrestrial animals, both for single organism model and for food webs. As an example, models can be found for earthworms, or some species of birds (such as seagull) and are generally scarce for other terrestrial species (Kelly and Gobas, 2003; Armitage and Gobas, 2007). Recent models are available for agricultural food chains to investigate the uptake process in cows (McLachlan, 1996). When dealing with uptake in plant biomass, it has to be considered that vegetation uptake was mostly investigated in the past 25 years, and several models are now available to predict root uptake and translocation to the aerial part and in the air to leaf path, especially for non polar chemicals. While root uptake is proportional to Kow, translocation is mostly limited to low range Kow chemicals (in the vicinity of Log Kow =2) and therefore uptake of more hydrophobic chemicals in leaves is generally driven by the air to leaf partitioning. Recent approaches were developed to quantify the so called "Forest filter effect" which depicts the forest capability to sequester chemical from air and transfer it to the soil environment and therefore to influence the soil ecosystem.

Filling the organism data gap

Mackay and Fraser (2000) reported an extensive list of needs, from the need to determine the extent of biomagnification of a variety of chemicals in a variety of species, metabolic half-lives (and the ability to predict them), reliable data on food consumption and assimilation, respiration rates, i.e. the basic bioenergetics of the organisms. As an additional remark, it was observed that field data are badly needed, given the necessity to test the models in realistic ecological situations.

While recent attempts were made to define and show the importance of some ecological parameters (LAI, SLA, temperature, precipitations etc.) in vegetation uptake, especially in characterizing the forest filter effect (Nizzetto et al, 2006a, b: Nizzetto et al. 2008a, b) a lot of work is needed to further define bioaccumulation behaviour and mechanisms for the variety of vegetal species, especially for herbaceous plants.

Filling the modelling gaps

Since most of the models available are based on a steady state representation of chemical uptake and losses and in a "constant" environment, there is a lack of unsteady-state or dynamic bioaccumulation models, designed to follow chemical concentration changes and organism lifecycle, from juvenile to adult stages, considering that such stages very often live in different environments (aquatic/terrestrial) and are consequently exposed to potential different concentrations. Such dynamic models are especially missing in the description of food web uptake, due to the complexity of data gathering and possibly the lack of knowledge of the fate modellers for ecological parameters and

cycles. Very few models (and for very few situations and organism) are currently available to describe the temporal accumulation of chemicals in the variable ecological conditions, especially when pulse exposure is present (De Laender et al., 2009). This is important for both aquatic and terrestrial ecosystems. There is also a need to build and validate food web models, especially those implementing mixed aquatic/terrestrial ecosystems to evaluate the contribution of chemical coming from different sources on the upper level of the food chain. Such models could be coupled with (dynamic) effect models to implement a "dynamic" risk assessment in a number of ecological situations.

Also PBPK models should be built for several representative organisms in order to understand and predict the temporal accumulation patterns of chemicals in organism.

There is also the need to develop approaches to evaluate the behaviour of polar/hydrophilic chemicals, protein-binding substances, and nanoparticles in biota.

While a number of ecological models is available, there is a need to connect exposure models (such as fate models including a dynamic biota compartment) to ecological models, in order to perform *ad hoc* simulations and evaluate/characterize the risk in more dynamic terms than the approaches available at present.

4.2.6. Exposure data and databases

In order to properly conduct exposure assessment, the source of data for any predictive approach is vital. The most important can be grouped as follows:

- physical-chemical properties and half-lives in environmental media
- environmental compartments characteristics
- mass transfer coefficients between compartments
- chemical emissions and temporal/spatial patterns
- validation/calibration/benchmarking datasets (different spatial and temporal resolution)

Major problems related with these data are discussed below.

<u>Needs for physico-chemical properties and half-lives</u>

Physical chemical properties (water solubility, vapour pressure, octanol-water partitioning coefficient, other solids/water partitioning coefficient, octanol/air partitioning coefficients, Henry's Law Constant or air/water partitioning coefficient, pK_a, etc.) and their temperature dependence are basic data required by the models. Problems lie in the number of chemicals for which such properties are available and reliable, albeit REACH should gradually fill the gaps, at least with the main physico-chemical properties. Also environmental and metabolic halflives (especially for air and sediment compartments) and their temperature dependence are generally scarce and often scenario or organism dependent. Related to this aspect biotransformation and biomagnification monitoring data for different classes of chemicals are needed to corroborate predictions and understand when current approaches are correct in predicting the bioaccumulation (e.g., Kow and lipid fraction in animals vs. protein binding chemicals).

Data for physical chemical properties are often scarce and uncertain. However, the implementation of the REACH Regulation is offering information on the physico-chemical properties of thousands of chemical substances. A summary of each relevant study is publicly available as well as the (Klimisch) reliability score. The information is published as presented by industry without a peer review by the authorities,

The European Chemicals Agency's Dissemination portal "ECHA Chem"[4] offers the specific information on chemical substances registered under REACH. The database already contains over 4000 substances and the number will increase for covering all substances manufactured or imported in the European Union above 1 tonne/year. This portal offers the information as submitted by industry. The evaluation of the existing and new information by the EU Member States may conclude the need for specific actions, such as proposal for classification, restrictions or identification of the substance as of high concern. The information assessed by public authorities under the REACH and CLP Regulations can be found through the ECHA Registry of Intentions[5].

The OECD Global information on Chemical Substances "eChemPortal"[6] compiles information on over 20 major chemical databases worldwide and provides free public access to information on chemical properties of chemicals. The information covers 19 general physical-chemical properties, 12 specific properties associated to the environmental fate and behaviour and the available monitoring, toxicity and ecotoxicity data.

As a complement, the OECD QSAR Toolbox[7] offers readily accessible (Q)SAR technology. The main features include the identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical; identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action; and the use of existing experimental data to fill the data gap(s).

Among the free tools available to estimate physico-chemical properties of chemicals, one is made available by the U.S. EPA (EPI Suite software[8]) and can be used to estimate a variety of properties for organic chemicals. Epi Suite was validated by a Science Advisory Board (U.S. EPA 2007) which provided a number of recommendations. The software was considered "scientifically defensible and appropriate for Agency regulatory screening applications" for chemicals similar to those for which modules to estimate chemical properties were developed, while for "existing and/or new chemicals whose structures and/or properties are outside the domain used in module development, scientific uncertainty may limit the utility of this software. In such cases, the Agency uses other methodologies to evaluate chemical properties". Additionally, properties for a number of broad chemical categories (e.g., polymers, organo-metallics, nanoparticles, etc.) were identified as impossible to predict by the current software version. The panel recommended increasing the investment in upgrading the algorithms for a larger range of chemicals.

Other data are available for pesticides under a publicly available web based database (The FOOTPRINT Pesticide Properties Database[9]) which was one of the products of an EU 6th Framework Programme project. It was developed by the Agriculture & Environment Research Unit (AERU) at the University of Hertfordshire. The database holds data (physical properties and ecotoxicological data for a range of taxa) for all EU Annex-1 listed pesticides and selected metabolites. The data come from a variety of sources; from monographs produced as part of the EU review process to other sources such as - databases and documents from various European government departments), U.S. EPA, and various on-line databases, manufacturers safety datasheets and environmental fact sheets, on- and off-line. Publications such as the Pesticide Manual, data derived from research projects as well as peer reviewed scientific publications were included. The data are 'tagged' with a code so that their source and quality can be identified. While the effort is valuable, problems arise from the wide variety of sources and quality of data, which often are not validated and/or not present in peer reviewed literature.

[4] http://echa.europa.eu/chem_data_en.asp
[5] http://echa.europa.eu/chem_data/reg_int_tables/reg_int_en.asp?substance_state=submitted
[6] http://www.echemportal.org/echemportal/propertysearch/page.action?pageID=0
[7] http://www.oecd.org/document/54/0,3746,en_2649_34379_42923638_1_1_1_1,00.html
[8] http://www. epa.gov/oppt/exposure/pubs/episuite.htm
[9] http://www. sitem.herts.ac.uk/aeru/footprint/en/

What transpires from the effort outlined above is the need for a consistent peer review of chemical properties and half-lives for existing and new chemicals, and the need for a general harmonization of procedures at international level to guarantee the quality of exposure assessment data.

Collecting realistic environmental compartments characteristics

The environmental compartment characteristics regard their composition and their temporal and spatial variability. In most of the regulative approaches these are fixed parameters characterizing "average" characteristics. Environmental temperature is usually considered among these parameters, as well as organic carbon fraction, fraction of air/water/solids/lipids, volumes, texture, etc.

These characteristics are generally contained in environmental fate models, user modifiable or "hard coded". They generally reflect average, annual or sometimes seasonal conditions. When a spatial pattern is present, geographical information systems (GIS) are often used to support the added complexity. The difficulty of obtaining environmental compartment characteristics lies in the lack of coherent datasets for specific systems, including their variation in time and space. Some data are present (sometimes as averages and not punctual numbers) in reports and datasets used in specific calibration validation exercises. The statistical information is also generally missing (averages are often lacking standard deviation or representativity of actual measurements) or the data measured during non routine monitoring campaigns are not generally available.

Assembling mass transfer coefficient databases for realistic scenarios

Mass transfer coefficients (MTCs) are specific inter-compartmental velocities used in compartmental models to predict the rate of transfer among phases. They are generally implemented in non equilibrium models and are referred to determined environmental conditions (e.g. a variable wind speed could determine variable air/soil mass transfer coefficient).

They are contained in environmental models and seldom are adapted to variable environmental conditions (such as wind speed, temperature, rainfall). A recent publication has collected and evaluated means to predict MTCs and their variations (Thibodeaux and Mackay, 2010). They recommend investigating MTCs for chemicals other than the traditional classes investigated (such as nanoparticles, dyes and pigments, ionizing substances) and for newly discovered phases (such as organic films on surfaces). Additional research is necessary to provide guidance on MTCs to be adopted in models to predict fate of chemicals in the extreme variety of scenarios and environmental conditions.

Obtaining chemical emissions and temporal/spatial patterns

Chemical emissions are key factors for the calculations of fate of chemicals because they determine the amount introduced in the environment. In steady state approaches (e.g. EUSES) they can be annual emissions (kg/y), while in dynamic approaches a temporal pattern should be specified (e.g. 2 kg/ha for the first 2 hours). Very often the temporal pattern is ignored or unknown. This is generally true also for the spatial pattern.

The implementation of the REACH Regulation is offering new challenges and possibilities for the emission assessment of industrial chemicals; based on the identification of the uses of each chemical and the exposure estimations.

The information on the used identified by the manufacturer/imported or communicated upstream through the supply chain by other users is part of the registration dossier and in general is made publicly available through the ECHA dissemination portal "ECHA Chem". Each use is codified using a descriptor system based on five separate descriptors,

which in combination with each other form a brief description of use. The sector of use category (SU) describes in which sector of the economy the substance is used. The chemical product category (PC) describes in which types of chemical products (= substances as such or in mixtures) the substance is finally contained when it is supplied to end-uses (by industrial, professional or consumer users).The process category (PROC) describes the application techniques or process types defined from the occupational perspective. The environmental release category (ERC) describes the broad conditions of use from the environmental perspective. The article category (AC) describes the type of article into which the substance has eventually been processed. This also includes mixtures in their dried or cured form (e.g. dried printing ink in newspapers; dried coatings on various surfaces). Some of the descriptor-lists support identification of the suitable Tier 1 exposure estimation tools. The environmental release categories [ERC] label the characteristics of a use based on six aspects relevant from the environmental perspective: the intended technical fate (purpose) of the substance during use; the life cycle stage; the dispersiveness of use; the application of contained application; indoor or outdoor uses; the use in articles handled under release-promoting conditions (such as abrasion). For all environmental release categories it is possible to derive a Tier 1 default (worst case) re-lease estimate (to air, water, soil) based on the release calculation module and the default release factors defined in the ECHA guidance on information requirement and chemical safety assessment (ECHA, 2008 and updates).

Exposure Scenarios for each identified use should be provided as part of the registration dossier for hazardous and PBT/vPvB substances manufactured or imported above 10 tonne/year and by downstream users who wants to include an additional use not covered in the registration dossier. In addition to the generic Tier 1 assessment tools, a set of Specific Environmental Release Categories (SPERCs) is currently under development.

The OECD Series offers 29 Emission Scenario Documents (ESDs) and 12 more under preparation.

The REACH Regulation has created additional requirement to industry for providing information related to the emission patterns of chemicals. The information is compiled through the REACH registration process and a complementary system of mandatory notifications, and covers three main elements: a database containing information on the uses, a compilation of (quantitative) exposure scenarios, and a set of notifications identifying the location of the main manufacturing and use processes.

The information on the used identified by the manufacturer/imported or communicated upstream through the supply chain by other users is part of the registration dossier and in general is made available to the public through the dissemination portal of ECHA "ECHA Chem". Each use is codified using five separate descriptors, which, in combination with each other, form a brief description of use: the sector of use category (SU); the chemical product category (PC); the process category (PROC), the environmental release category (ERC) and the article category (AC). The combination of the five descriptors may provide the title for the exposure scenario.

The Exposure Scenarios are part of the Chemical Safety Report provided with the registration or the notification from downstreams users which incorporate a new use. The exposure scenarios are not disseminated by ECHA; but should be attached to the Safety Data Sheet and therefore are disseminated through the supply chain.

The information on the location of manufactures and their main uses, uses subjected to authorisation, and the information on the downstream users which include a new use or receives an authorised substance is only available to public authorities. Nevertheless it constitutes a unique possibility for creating a georeferenced database with the location of the main site-specific emission sources.

The combination of these three pieces of information offers huge possibilities for developing tools for improved site-specific and region-specific aggregated exposure estimations. The descriptor system can be used for the identification of substances which share similar uses and/or emission patterns. As described before, for all environmental

release categories it is possible to derive a Tier 1 default (worst case) release estimate (to air, water, soil) based on the release calculation module and the default release factors. The location of the main release point sources would allow the adjustment of the default values to the specific environmental conditions of the receiving site and a combined assessment of point emission estimations within a particular area, e.g. a river basin. The use descriptor system also allows the identification of widely dispersive uses, e.g. consumer uses with releases associated to municipal waste and wastewater treatment, which contribution can be added on the based on the population distribution/structure in the area.

Apart from pesticides, emissions are often available as general yearly discharge in a medium and therefore a temporal profile of such emission cannot be used. EU has implemented on line registries such as EPER [10] , which was replaced by E-PRTR [11] but such database contain declared emissions at annual level for a large number of facilities and sectors in Europe. While such numbers are precious to estimate the order of magnitude of discharges in a certain region, they lack in temporal and often spatial resolution. Also the number of chemical present is small (91 chemicals at July 2011).

Therefore, the capability of creating spatial and temporal varying emission scenario for modelling purposes is rather scarce or very generic. In order to provide higher tier predictions, a higher dynamic of emission pattern would be necessary.

Obtaining validation/calibration/benchmarking datasets (different spatial and temporal resolutions)

Validation/calibration/benchmarching datasets are databases of environmental/ measured chemical concentrations for a certain territory and are used in the so called process of calibration and "validation" (also called bench-marking) of models.

Monitoring data, especially for "recent compounds" are scarce as well as "validation" data sets (with a multimedia array of concentrations in a variety of phases) for specific purposes (e.g. surface water, soil, multimedia environments, global situations) and for discharge patterns (continuous to seasonal and pulse). Their availability is usually limited to specific publications or reports. While more data exist for local or site specific situations (e.g. for chemicals directly applied to soil or water such as pesticides), they are very scarce for more general widely-used chemicals such as those regulated with EUSES. It becomes therefore very difficult to compare predicted and measured concentrations, especially when the discharge compartment is very different from the one predicted or the chemical is poorly mobile. Additionally, very few datasets encompass an entire year and few span through multiple years. Time resolution, is also problematic: the best resolution is often daily, while some environmental phenomena can be very rapid and would require the evaluation of concentration variations at shorter (e.g. hourly or less) time intervals.

4.2.7. Comparing measured and predicted exposure data

The final assessment of ecological exposure may be performed trough a comparison between measured and modelled data that is currently largely missing. However this should be made very carefully in order to avoid comparing figures that are not homogeneous. For a thorough analysis, the following issues are important:

1. In many cases the modelled results are partly based on measured input data (emission rates, partition coefficients, concentrations in specific environmental compartments, etc.). The more measured data are used, the more the representative measured and final model results should agree.

[10] http://eper.ec.europa.eu/eper/
[11] http://prtr.ec.europa.eu/

2. Environmental concentrations are highly variable due to several factors (emission patterns, environmental characteristics, etc.). Therefore, measured data should be representative for the exposure scenario considered. Model results are also affected by uncertainty or variability in substance-specific parameters, scenarios and models.

3. Modelled results usually only consider exposure from specific anthropogenic emissions in the life cycle of a substance (i. e. production, processing and use). Measured data also depend upon unintentional and natural emissions. Background levels may be caused mainly by such sources, so natural background levels should be taken into account in risk assessment and risk management.

4. For a proper comparison between measured and modelled data, a thorough analysis of the representativeness and quality of the measured data, the nature of the exposure model scenario and the uncertainty in both the measured and modelled results is necessary.

4.3.Assessment of effects

4.3.1. State of the science

The methodology used in ecotoxicology has been borrowed almost directly from toxicology: measurements of effects (on survival, reproduction and development) in a few individuals of a few test species. Yet the goals are different, since individuals are rarely important in ecological protection (Hommen et al., 2010). This is because individuals can be removed from populations without necessarily affecting the size and dynamics of these populations and the communities of which they are part. Hence, in ecological protection the extrapolation problem in assessing risk is often from observations on impacts on a few individuals in a few species to likely impacts on the populations of many species and their consequences for ecosystem structure, processes and services (cf. human health assessments where individual humans matter and the extrapolation is often from a few laboratory test species to one).

From a regulatory perspective, the ecotoxicological outputs are used to support ecological risk assessments. There are two main kinds of regulatory instruments for chemicals in the environment. The first kind, such as REACH and the authorization instruments for plant protection products and biocides, seek to anticipate likely effects of chemicals on ecosystems. In these regulatory instruments, the protection goals are only vaguely defined, e.g. as "safe use in terms of human health and the environment". However, the guidance documents that accompany them make it reasonably plain that it is populations and ecosystems that are of concern. The other kind of instruments, such as the Water Framework Directive (EC, 2000), the Marine Strategy Framework Directive (EC, 2008) and the forthcoming Soil Directive, seek to protect an overall ecological quality status defined in terms of populations and ecosystems.Other, more generic regulatory instruments should also be considered. Typical examples are regulatory provisions for preventing and remedying industrial emissions, such as the IPPC, Seveso II or Environmental Liability Directives; and those focusing on the protection of habitats and biodiversity, such as the Birds and the Habitats Directives. The focus of these complementary instruments tends to be holistic, covering all kinds of hazards, but when the main risk is related to chemical substances, their implementation creates new challenges and opportunities for ecotoxicology, requiring a site-specific or region-specific effect assessment associated to the particular ecological values of the area considered (Macedo-Sousa et al., 2009).

Notwithstanding the complex requirements of ecological risk assessment, reductionist methods have had some success in ecotoxicological research because they offer the promise of bringing in fundamental science to address ecological problems and hence offer the prospect of funding opportunities. Yet methodologies based on biomarkers and omics have made little practical contribution to the development of more ecologically

relevant risk assessments that can be useful and used routinely in a regulatory context. By contrast there has been much discussion on the importance of addressing the "eco" in ecotoxicology explicitly when developing more relevant ecological risk assessments but there has been generally limited action. This has been partly due to the inaccessibility of the ecological theory for application, and in some reticence in the regulatory community to move away from well-tried methodologies and approaches. One possible consequence of this is that uncertainties in the extrapolations from the standard tests are treated somewhat conservatively.

Recent developments, involving collaborations between, academic, regulatory and regulated communities are seeking to develop more user-friendly approaches to the use of models in ecological risk assessment. These ecological models have the potential of not only for making predictions about relevant effects as a basis for risk assessment but also of indicating what kinds of measurements should be made on what kinds of species as a basis for the ecotoxicological work (Forbes et al., 2009). The challenge will be to develop models that are sufficiently rigorous to satisfy the requirements of the theory but also robust and transparent enough to be put into practice on a routine basis and that command the confidence of the regulatory community.

It must be also considered that the need for new tools in ecological risk assessment is also a consequence of the changes in environmental problems and pollution in the last decades. Up to the 1970s, chemicals in ecosystems, in particular in surface water, were likely to produce effects at the sub-acute, sometimes even at the acute, level on natural populations. Therefore ecotoxicology was developed with the aim to develop tools capable to quantify the risk from chemicals and was mainly based on testing approaches for the assessment of dose/concentration-effects relationships.

The increased level of chemical control, occurred in all developed countries starting from the 1970s, led to a substantial reduction of these severe effects. As a consequence, there is an increasing need for approaches capable to answer to more complex questions than dose/concentration-response relationships can. To do this, it is essential to improve the predictive power of ecology and ecotoxicology for describing effects at the hierarchical level of communities (Van Straalen 2003).

4.3.2. Emerging issues

Complexity of the issues

As mentioned above, the main question posed by chemical regulations (e.g. REACH, Pesticide Directive, etc.) about ecological risk assessment is: may a given chemical be put in the European market without unacceptable risk for the European environment?

For other kinds of environmental regulations ecological risk assessment may be applied in order to solve other kinds of problems related to the development of appropriate management plans for the protection of environment and natural resources that cannot exclude the potential effect of chemical emissions. All these pieces of legislation require tools capable to accounting for the characteristics of specific ecosystems.

We must be aware that the complexity of the problem is two-fold:

1. *the complexity of biological communities*: the characteristics of a community are not merely the sum of the characteristics of individual populations; structure and function of the community is regulated by emergent properties that are not easily described and predicted from lower hierarchical levels;
2. *the complexity of stress factors*: toxic agents are only one component among a multitude of potential stress factors that may alter the behaviour of natural populations and communities; the combination of multiple stress factors (anthropogenic and natural) needs to be taken into account for explaining environmental changes.

To address these issues, some more advanced tools have been recently applied in ecological risk assessment; however, their potential has not yet been fully explored. Moreover, some ecological concepts, necessary to understand actual effects on structure and functions of ecosystems, have never been used in ecological risk assessment procedures. The challenge for the use in ecological risk assessment, particularly for regulatory purposes, is the development of suitable tools capable to produce quantitative information and to be applied in a transparent way. Even though some excellent scientific contributions have been made in the last few years, there is still a strong need for research in these fields.

The use of higher tier approaches such as mesocosms and model ecosystems should be implemented. However, the results should be used with care as the model ecosystem used may not necessarily reflect the situation in the field (e.g. as environmental parameters may differ). Hence, methods for assessing the representativeness of the tools used and their possibility of extrapolation to different ecosystem typologies must be developed. One way to enhance the development and explore the use of these methods may be to use existing mesocosm data sets and new modelling tools.

The Species Sensitivity Distribution (SSD) method, although it has some limitations, is a powerful tool for assessing effects on community structure. At present, it has been used in risk assessment procedures (e.g. Maltby et al., 2005). However, its application is often impossible due to the scarcity of data. Moreover, the effects on ecosystem functioning must be better assessed not only on the basis of the number of species potentially affected, but also considering on the degree their ecological role is affected.

The SPEAR approach links exposure to ecosystem effects considering physiological sensitivity of species and traits relevant to toxicant effect (Liess & von der Ohe, 2005). The approach has proved to link steadily exposure and community composition in various ecosystems (Schäfer et al. 2007). The approach additionally predicts interactive effects of environmental stressors and toxicants as well as indirect effects of pesticides within the ecosystem context (Liess & Beketov 2011). Its ability to detect effects of pesticides alone in relation to most environmental stressors is established (Liess et al. 2008). However, the SPEAR approach lacks the possibility to discriminate the effects of pesticides simultaneously applied.

A conceptual model for developing realistic risk assessment approaches should be based on knowledge of the community structure, assessment of different exposure patterns for the major ecological and trophic roles, interactions among biological, physical and chemical factors (Macedo Sousa et al., 2009). Many of these issues are described with more detail in the following sections.

The need for more ecologically realistic approaches is particularly relevant for site- or region (ecoregion)- specific risk assessment. An open question is whether it is possible to expand these types of realistic site/region-specific assessments to generic/regional assessments covering the true variability of environmental characteristics in a given area. There is thus a clear need to define overarching (fundamental science-based) ecological risk assessment principles which can support these tools. More research on the development of these kinds of approaches may allow developing tools capable to describe the spatial distribution of ecological risk, for example by using GIS-based risk maps at different scale levels, from local to continental (Sala and Vighi 2008; Pistocchi et al. 2011).

Some relevant issues that may be useful to increase the ecological realism of effect assessment approaches are listed below. They include processes that need to be better understood and described, as well as tools that need to be developed to reach these objectives. All issues are developed in detail in the sections below.

a) Processes

- Assessing the effects of time-variable exposure

- Covering the gap between effects at cellular or individual level and their consequences for ecologically relevant endpoints.
- Assessing the vulnerability of populations, communities and ecosystems
- Identifying the relevant and desired protection level of ecological systems.
- Assessing the role of indirect ecological effects of stressors
- Assessing the interactions between toxicants and environmental factors.

b) Tools

- Trait-based risk assessment.
- Improving the scientific bases for the development of extrapolation approaches
- Ecological modelling

Assessing the effects of highly time variable exposures

Most ecotoxicological data refer to constant or regularly changing exposure. However, this is not the case in realistic exposure of natural ecosystems.

Aquatic organisms are often exposed to variable and/or sequential pulses of chemical stressors with fluctuating concentrations; this may occur, for example, when pharmaceuticals are discharged through sewage treatment plants (which may vary with time during a day) or when pesticides reach water via various pathways, (which are driven by rainfall events) (Reinert *et al*. 2002). These exposure patterns call for an assessment of time-dependent and delayed effects of pulsed toxicant exposure. Delayed effects may result from two mechanisms: (1) direct delayed effects on the individual level where the effect of a toxicant is also apparent even a long time after exposure (Liess, 2002) and (2) indirect delayed effect where competing individuals or species are delaying recovery of the affected species (Liess & Foit, 2010).

Standard toxicity tests with aquatic organisms are performed either at steady concentrations (flow-through), under semi-static conditions (periodic inputs of material) or under static conditions (single initial input of test material). Hence, any extrapolation to more realistic patterns of exposure must rely either on - experiments designed specifically for specific exposure scenarios or on modelling (Boxall *et al*. 2002). Modelling has the advantage that one can extrapolate to a wide range of field exposure scenarios. A variety of approaches has been developed (Reinert *et al*. 2002; Ashauer *et al*. 2006), and the importance of the response by individual organisms to recovery periods between successive pulses has been recognized (Kallander *et a*l. 1997; Ashauer *et al*. 2007).

Toxicokinetic / toxicodynamic (TK/TD) models describe the processes that link exposure to effects in an organism; available models were reviewed by Ashauer *et al*. (2006) and Brock et al. (2009). Toxicokinetics consider the time course of concentrations within an aquatic organism in relation to concentrations in the external medium. The simplest description of toxicokinetics is the one-compartment first-order kinetics model which is also the most commonly used in aquatic ecotoxicology. It describes the dynamics of the internal (whole body) concentration of the toxicant C_{int} depending upon the external concentration C_{ext} using uptake and elimination rate constants (k_{in}, k_{out}) and the external concentration. Toxicodynamics describe the time course of damage and repair to the affected organisms based on specific pattern(s) of exposure to the test compound. Models differ in their assumptions about toxicodynamics, but most are hazard-based and assume that death (or a sub-lethal endpoint), although depending upon the toxicant concentration in the organism or the damage, is at least partly stochastic (Ashauer et al., 2006).

After selecting a model that simulates effects in an organism based on the time course of the contaminant concentration, the model parameters are estimated by calibration on experimental data, and the model performance is evaluated against independent

experimental data. This establishes a range of validity within which the model can be used to extrapolate to effects from specific patterns of exposure. Uptake-elimination experiments determine the time course of concentrations within an aquatic organism in relation to concentrations in the external medium. Generally the work is undertaken to derive model input parameters that will allow prediction of internal concentrations of chemicals for new situations. Toxicokinetic parameters may vary with life stage of a species, primarily in response to changes in lipid content. Toxicodynamic experiments infer the time course of damage and repair to the target organisms based on the time-course of survival in response to specific pattern(s) of exposure to the test compound. The information can be used to estimate toxicodynamic parameters within TK/TD models. None of the TK/TD models has been extensively validated to date. Use of the models for risk assessment should thus be supported with validation experiments for the particular combination of compound and organism. Ideally, validation experiments should include an exposure profile that contrasts markedly with those used in model calibration (e.g. more/less pulses of shorter/longer duration than previously tested). Longer-term experiments are also useful to demonstrate the ability to extrapolate beyond the precise conditions of the calibration experiments.

A major advantage of the TK/TD modelling approach is that it should not be limited in terms of the kind of exposure profile which can be considered. Most of the effort is required in undertaking experiments to determine and evaluate model input parameters that are robust and broadly applicable. Once this has been done, the models are quick to run and predictions for effects can be generated for a large number of exposure situations.

TK/TD modelling for effects of pesticides on aquatic organisms has been primarily a research activity to date. The approach is best developed for aquatic invertebrates at present. For relatively simple animals, the assumption of uniform internal concentration appears to hold and the toxicokinetics can be simulated with a single-compartment, first-order model. A major constraint to current models is that they apply in situations where the duration of exposure is less than the duration of life of the test species and generally assume negligible growth and negligible change in lipid content during the period of exposure (such conditions might reasonably by applied to *Gammarus* and *Asellus*, for example). There is no intrinsic reason that the models cannot be developed to account for species with short generation times where growth and/or reproduction are significant within the duration of exposure. However, further work is required on this aspect, especially for organisms with a more complex life cycle like insects.

The methodology has generally been applied to effects on survival. However, there is no intrinsic limitation to use of the approach with sub-lethal endpoints (e.g. growth or reproduction) provided that the model has been appropriately calibrated on the respective endpoint. TheTK/TD models may require a limited amount of modification to be applicable to non-lethal endpoints, but the overall conceptualisation of the system remains the same. The DEB theory may be particularly suited to simulation of sub-lethal effects that result from changes in energy allocation within the organism because energy usage and budgets within organisms are explicitly simulated.

There may be significant inter-species variability in toxicokinetics and/or toxicodynamics for a particular chemical. The extent of this variability needs to be quantified and decisions on utility in risk assessment, selection of representative organisms etc. guided accordingly.

TK/TD modelling has also been applied to simulate effects of toxicants in fish (Jager & Kooijman, 2005 for AChE inhibition). Generally speaking, model complexity increases with respect to the toxicokinetics because of the need to model partition and depuration processes for individual compartments within the organism. Given the intensive data demands at the parameterisation stage, it seems unlikely that these models will be extensively applied to vertebrates because of animal welfare concerns.

Hardly any work is available on simulation of effects of time-varying exposures on aquatic plants and aquatic communities. Extension to plants with simple structures seems relatively straightforward. Preliminary experiments comparing effects on *Lemna minor* from constant and time-varying exposure to six pesticides have been reported by Boxall *et al*. (2005). Zafar et al. (2011) describes one of the first studies evaluating the effects of time-varying exposure to pesticides on the ecology of aquatic model ecosystems.

Covering the gap between effects at cellular level and consequences for ecologically relevant endpoints

Effect data at lower hierarchical levels (e.g. cellular) such as biomarkers and omics-based endpoints are at present, of little direct relevance to and not useful in ecological risk assessment. However, they may have a potential as early warning tools of exposure and effects to come. It should be evaluated to which extent a link between these effects and the responses at population level is possible. Genomics technology, especially transcription profiling, allows new ways to assess biological effects of environmental pollution. The basic idea is that gene expression is one of the very first things that will change when an organism is exposed to a stressful condition. To maintain homeostasis of the internal environment, the metabolic machinery requires continuous adjustment to any new situation. Gene expression change is a direct reflection of these adjustments. Because of the potential advantages, several regulatory authorities are now discussing how genomics tools could fit into the risk assessment process. The US Environmental Protection Agency is developing new guidance that outlines how genomics may contribute to a weight-of-evidence approach towards assessing environmental pollution (Dix et al. 2006).

Transcription profiling as an environmental monitoring tool has some advantages over traditional bioassays that focus on survival, growth and reproduction of test animals. Three possible benefits have been outlined (Van Straalen and Roelofs 2008):

(i) gene expression will be specific to the type of stress, unlike classical endpoints such as growth and reproduction,

(ii) gene expression will be more sensitive, that is, effects can be recognized at lower exposure concentrations, compared to classical endpoints, and

(iii) gene expression will respond quickly, in the order of hours to days, allowing tests that otherwise may take several weeks.

These claims have not yet been substantiated, however, several pioneering studies are now being published. These create a basis for testing these assumptions and evaluating the high expectations raised. These preliminary studies suggest that claims (i) and (iii) have a solid basis in experimental work, and that claim (ii) may be less well supported. Gene expression profiles bear a strong signature of the mode of action of the toxicant and can be observed to change already after hours to days, when nothing special is observed on the phenotypic (whole-body) level. In other words, the strongest advantage of gene expression profiling is that it is quick and specific.

For the near future three challenges need immediate attention, if we want to forward ecotoxicogenomics from its still infancy stage to full-fledged maturity.

(i) Distinguish fitness-neutral gene expression from gene expression indicative of adverse effects. Because of the principle of homeostasis, any animal constantly adjusts its internal processes to deal with changing environmental conditons or internal needs. Most of these gene expressions belong to the normal range of operation and have nothing to do with toxicants. Consequently we must find classifying systems that allocate gene expression changes under normal operation from those associated with adverse effects. The concepts of "NOTEL (no-observed transcriptional effect level)" and "adverse outcome pathway" may be useful in this respect (Poynton et al. 2008; Van Aggelen et al. 2010).

(ii) Distinguishing gene expression changes indicative of chemical exposure from gene expression changes indicative of adverse effects. Most of the efforts in ecotoxicogenomics studies has gone to demonstrating gene expression change over short exposure periods. The profiles observed in such studies are closely related to the mode of action of the substance and they demonstrate the presence of substances of certain toxicological properties in the environment, but they are not necessarily predicting phenotypic effects appearing after longer exposures which are the endpoints of classical toxicity tests. How these two categories of gene expression are related to each other is not yet known.

(iii) Filtering substance-specific gene expressions from those induced by mixtures of compounds. The few ecotoxicogenomics studies that have considered mixtures suggest that mixtures may induce other genes than either of the constituent chemicals. On the gene expression level, a mixture appears like a new chemical. Whether it is possible to recognize substance-specific profiles from mixture effects is not known.

Assessing the vulnerability of populations, communities and ecosystems

Vulnerability of populations, communities and ecosystems is the result of the combination of four different components (Ippolito et al., 2010; Van den Brink, 2008):

- the susceptibility to exposure
- the sensitivity to the specific stressor
- the recovery capability (resilience)
- the indirect ecosystem responses.

Vulnerability is not accounted for in the currently used procedures of ecological risk assessment. However, in nature vulnerability of these biological systems is not constant. Instead vulnerability is highly variable within the natural context because of two processes.

First, environmental conditions are driving the structure of biological systems as populations and communities. This is not only that species are distributed according to their niche within the combination of environmental factors. Also the frequency of disturbance typical for a specific ecosystem governs the distribution of traits which are relevant for the vulnerability against episodic exposure. For example it is long known that ecosystems that are subjected to regular disturbance support a higher proportion of short lived organisms characterised by high population growth rates and high recovery potential. Hence, it is obvious that especially those species are showing a faster recovery after pulsed (e.g. pesticide) exposure as shown in mesocosm- (Beketov et al., 2008) and field monitoring investigations (Liess et al. 2008).

Second, stress conditions often increase the sensitivity of individuals and populations to toxicants (see Heugens et al. 2001 for review). Hence, to determine the quantitative link between exposure and effect it is necessary to consider additional stress factors. Such factors include a wide range of stressors that increase the rate of mortality due to toxicants: exalted temperature (Song et al. 1997), food limitation (Pieters et al. 2005), exalted salinity (Wildgust and Jones 1998), low oxygen (Van der Geest et al. 2002), UV radiation (Liess et al. 2001), competition (Liess 2002), predation (Beketov and Liess 2006) and requirement of food acquisition (Mommaerts et al. 2010). Especially for protected and endangered species that are generally subject to stress within their environment these process will lead to an increased sensitivity within the environmental context (Liess et al 2010).In summary, we need to consider that ecosystems and communities developing under more constant conditions may show rather slow recovery rates from pulsed toxicant exposure than those living under more variable conditions. Additionally, individuals, populations and communities that are stressed by environmental conditions may show increased sensitivity to toxicants.

Identifying the relevant and desired protection level of ecological systems

As highlighted above, the objective of ecological risk assessment is the protection of structure and functions of biological communities and ecosystems. Some pieces of legislation provide also indication of the extent of ecosystem protection to be attained. According to the WFD, the objective is the attainment of a "good" ecological status defined as follows (EC,2000):

"The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water-body type under undisturbed conditions."

In other words, the characteristics of aquatic ecosystems should be as close as possible to the reference conditions of natural water bodies not subject to human pressure.

This kind of definition is not applicable to other kinds of ecosystems, particularly in the terrestrial environment where human pressure fully changed the structure and the characteristics of ecosystems that may not be restored at levels close to "undisturbed conditions". For example, in intensive agricultural areas, a new type of ecosystem (the agro-ecosystem) is established and the objective of protection must be adapted in order to preserve structure and functions of this system that is the combined result of natural and human pressures. A specific objective for agro-ecosystems could be the protection of "Ecosystem Services" (e.g. the functioning of the soil community, the activity of pollinators, etc.), by accepting an anthropocentric concept of environmental protection (Nienstedt et al., 2012).

Conversely, a special level of protection should be applied in Specially Protected Areas (SPAs). In these areas risk assessment approaches must consider the high ecological value and, in many cases, the high sensitivity and vulnerability of the ecosystems. In particular, different approaches must be developed for highly endangered species that may have to undergo specific risk assessment with the identification of effects at the individual level. However it must be noted that, even in this case, the objective, unlike for human health effects, is the protection of individuals in order to attain an ecologically acceptable size of the population.

Assessing the effects of particularly ecologically relevant chemicals (e.g. EDC).

According to the IPCS/OECD definition (EC, 1999):

"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

The effects of an endocrine disrupting chemical (EDC) may produce alterations to reproduction, growth and development patterns, affecting population dynamics. Therefore, according to the objectives of protection described in the previous section, endocrine disrupting effects must be considered as highly ecologically relevant.

In order to study the potential endocrine disrupting effect of a chemical some knowledge is needed on the endocrine system of the target organism and on its effects on reproduction and development. However, present knowledge is mainly focused on vertebrates, particularly mammals.

One of the most deeply studied endocrine effect in invertebrates is imposex produced in molluscs by organotin compounds (particularly tributhyltin, TBT). Imposex occurrence in many species of marine molluscs is widely documented in the literature (see, for example, Fioroni et al., 1991; Huet et al., 1996). Some mechanisms of endocrine systems of insects, mainly related to moulting and metamorphosis, have been studied in

relation to the development of specifically acting insecticides. However, besides these studies, few is known on endocrine systems of invertebrates.

Currently used ecotoxicological tests are not effective for assessing potential endocrine disrupting effects. Even reproduction-oriented tests (e.g. *Daphnia* reproduction test) are not suitable for producing this kind of information. A general, non-specific stress condition may produce impairment in reproduction efficiency. Assessing endocrine effects and classifying chemicals as EDC would require more specific approaches. As stated by CSTEE in its opinion endocrine disrupting effects (EC, 1999):

"an endocrine disrupter can only be defined in a complete organism containing a functional endocrine system. The ecological significance of such a compound needs to be demonstrated in a free-living animal exposed in its natural habitat. Consequently, a full understanding of a health effect resulting from endocrine disruption in a wild population requires a combination of experimental, mechanistic studies under controlled exposure conditions and an association between a health effect and exposure to a defined chemical or group of chemicals in the real world."

Considering the complexity of the issue and the relevance of endocrine disruption for ecosystem protection, the improvement of knowledge on endocrine systems in invertebrates and the development of procedures for assessing endocrine disrupting effects and for classifying EDC represent a relevant priority for research.

Assessing the role of indirect ecological effects of stressors

Immediately following their release into the environment, the effects of a chemical on the species in an ecosystem depend on the sensitivity of these species for the chemical. These effects can be termed 'direct effects' and form the basis of today's risk assessment procedures. Relations between (e.g. interspecific competition, predator-prey) and within populations (e.g. intraspecific competition) may cause (positive or negative) 'indirect' effects (Fleeger et al. 2003, Relyea and Hoverman, 2006). These indirect effects are more difficult to predict than direct effects, given the complexity of the relations within an ecosystem and environmental constraints such as nutrient status (e.g. Petersen et al., 2009). Indirect effects are therefore one of the key problems that hamper an accurate prediction of chemical effects in the field.

Influence of predation and competition on recovery

The ecotoxicological impact of a toxicant depends on the magnitude of the effect and on the potential of affected populations to recover. The duration of the recovery process of a population also determines the extent to which a toxicant indirectly affects the community. Hence, the ability to predict the duration of recovery determines our ability to predict the extent of toxicant effects on an ecosystem. In the following three different ecological scenarios are distinguished that differ in recovery dynamic: recovery of an isolated population, recovery of a population in competition with another species, recovery of a population under predation.

Isolated population close to carrying capacity that are not subject to competition or predation by other species are characterised by a high degree of intra-specific competition. A reduction of abundance as a result of exposure to a toxicant reduces intra-specific competition as more resources for survivors are available. Recovery of populations can be assessed by integrating endpoints that describe a system in its entirety (e.g. total abundance or biomass), or differentiating endpoints that describe a system by grouping its elements according to relevant traits (e.g. population structure as size or age distribution) (Liess and Foit 2010). Recovery of a population in competition with another species, were the community is close to a dynamic equilibrium, is characterised by a high degree of inter-specific competition. A reduction of abundance, as a result of exposure to a toxicant, reduces inter-specific competition as more resources for survivors are available. In case the competing species is less sensitive or is

characterised by a higher population growth rate, this will recover faster and reduce recovery of the first species. Such indirect effects were observed for example in a field investigation quantifying the effects of pesticides on invertebrate communities (Liess & von der Ohe, 2005). Within filed test systems it was reveald that competition with a less sensitive species increases recovery time by a factor of more than 3 (Knillmann et al. 2012).

Recovery of a population under predation is rarely investigated. One of the few existing publications shows that artificial predation prolonged time for recovery and increases risk of extinction (Beketov & Liess 2006). Additionally it was shown that low toxicant concentrations may act by reducing alarm responses of the prey. This inhibition may increase larval mortality due to predation (Reynaldi et al. 2011).

How can indirect effects on ecosystem structure and functions be quantified?

Studies dealing with indirect effects often consider abundance or biomass as endpoints, i.e. proxies for ecosystem structure. Examples of such effects include (1) an increase of phytoplankton density (Friberg-Jensen et al., 2003) following reduced grazer density; (2) a reduction of fish population size following reduced grazer density (Boyle et al., 1996); and (3) the dominance of small zooplankton following the decimation of larger grazers such as *Daphnia sp*. (Hanazato, 1998). Apart from such 'second order' indirect effects, also higher order indirect effects have been observed in which the chain of effects is longer (Relyea and Diecks, 2008; Relyea and Hoverman, 2008).

Fleeger et al (2003) give an overview of indirect effects reported in 150 studies with experimental ecosystems. The most common type of observed indirect effect appears to be an abundance increase of primary producers following grazer elimination by contaminants. Indirect effects in the form of abundance reductions are much less frequently reported. For example, Liebig et al. (2008) showed that protozoa were reduced in numbers at concentrations that were orders of magnitude lower than their single-species NOEC, allegedly because of food shortage after their prey (algae) was decimated by the herbicide promethrin.

Besides experimental approaches, also mathematical modelling of ecosystems has been used to quantify chemical effects on ecosystem structure (De Laender et al., 2008; Naito et al., 2003). These models typically describe population growth of connected species taking into account interactions between species such as predation, competition and density dependence. A recent theoretical study using such a model examined how many species in an ecosystem would suffer a population biomass decrease if a predefined number of them was affected directly by growth rate reduction (De Laender et al., 2010a). This study showed that the number of species experiencing biomass reduction is nearly always smaller than the number of directly affected species. This suggests that for a number of directly affected species, these direct effects are compensated by positive indirect effects. These simulations provide an additional indication that risk assessments that are solely based on single-species toxicity, i.e. on direct effects, may overestimate the effects occurring in ecosystems (Selck et al., 2002; van Wijngaarden et al., 2010; Versteeg et al., 1999).

Much less attention has been paid to indirect effects on ecosystem functions than to indirect effects on ecosystem structure. As a result, too few studies that have measured ecosystem functions in contaminated micro-or mesocosms (e.g. Carman et al., 2000; Kersting and Van Wijngaarden, 1999; Koshikawa et al., 2007) may have been performed to obtain a general conclusive view on this matter. Recently, inverse models have been applied to infer chemical effects on carbon flows using available micro-or mesocosm data (De Laender et al., 2010b). These exercises have revealed indirect functional effects of an insecticide (De Laender et al., 2010b) but not of a herbicide (De Laender et al., 2011).

Conclusion

The available data suggest that indirect effects on ecosystem structure mitigate the direct effects caused by chemicals in some cases. In other cases indirect effects due to a change in competitive balance increases effect and decreases potential for recovery. The future challenges include the further development of quantitative techniques to predict the negative indirect effects on ecosystem structure. For ecosystem functions, it is uncertain under which circumstances indirect effects are positive or negative. Clearly, more work needs to be done, possibly by revisiting currently available data using novel techniques.

Assessing the interactions between combined stressors and environmental factors

International chemical regulations are based predominantly on assessments carried out on individual substances. However, organisms are usually exposed to several stressors.

The issue of the combined effect of chemical mixtures is now under specific consideration by the European Commission. A recent scientific opinion answers a number of questions in support of the development of regulatory proposals (for human health and environment) for the control of chemical mixtures (SCHER, SCCS, SCENIHR, 2011). It could provide some more precise elements for deriving risk assessment procedures for chemical mixtures by applying different approaches as a function of the amount of information available on mixture composition, toxic effect and toxicological modes of action of individual chemicals.

However, chemicals are not the only potential stressors. Many other factors may affect structure and functioning of biological communities, and may interact with the toxic effects of chemicals (e.g. temperature, oxygen depletion in aquatic ecosystems, water shortage in soil, etc). Nevertheless, virtually all current methodologies for ecotoxicity testing are based on simple, usually short-term tests, performed at standardized laboratory conditions. These "standard conditions" mean that animals are kept at constant environmental conditions (e.g. temperature), being either fed *ad libitum* or starved. These are certainly not the conditions that animals are usually exposed to in the field, where large fluctuations in climatic conditions and food availability are the norm, and where huge differences can be found across geographical scales.

Indeed, a number of studies have shown that all these natural environmental factors can significantly affect the effects of toxicants on organisms. Already more than 25 years ago Bryant et al. (1985) noticed that salinity and temperature may significantly affect toxicity test results. This was especially clear in the clam *Macoma baltica*, in which the median survival time at the very same concentration of nickel could be as low as 50 hours or as long as ca. 300 hours, depending exclusively on the combination of water salinity and temperature. This led the authors to state that "*the environmental variables of temperature and salinity should be considered when evaluating toxicity of nickel and zinc in the estuarine environment*". In the same year Demon and Eijsackers (1985) published a paper on effects of extreme temperatures, temperature fluctuations and moisture on toxicity of two pesticides to two species of soil invertebrates: an isopod and a springtail. It appeared that the combined effect of the pesticides and high temperature was synergistic, and the springtail was also more susceptible to the pesticides at desiccating conditions. Consequently, the authors concluded that unfavorable environmental conditions must be incorporated into assessment procedures. To date, this has not been done.

More studies on interactions between toxicants and natural stressors/suboptimal conditions have been performed in the last 20 years but they have never been in the mainstream research topic in ecotoxicology, and these findings have never made their way to ecological risk assessment and testing of potentially toxic chemicals. It is worth noticing that, unfortunately, the interactions between natural stressors and toxicants are very difficult to predict as they can differ vastly even among closely related species, as

shown by Holmstrup et al. (1998) for desiccation effect on copper-induced mortality in earthworms. As shown more recently by Bednarska et al. (2009), in some cases also higher level interactions (that is, between more than just two factors) are highly significant and equally highly unpredictable. The meta-analysis of 61 studies performed under the umbrella of EU funded project "NoMiracle" (6th FP) showed significant interactions between chemicals and temperature in 62% cases, moisture/humidity in 51% cases, and dissolved oxygen in aquatic environments in 100% cases.

in conclusion, significant interactions between toxic chemicals and natural stressing factors under suboptimal conditions have been proven and appear to be a rule rather than an exception. Due to their magnitude, many are not only statistically significant but seem also important from the point of view of species biology, population dynamics and, thus, for ecological risk assessment. Interactions with natural stressors should be, thus, incorporated in ecological risk assessment. Such tests require, however, much more complicated and elaborated test designs. Hence it is not practical to perform them routinely for all tested chemicals. It is suggested that most important natural factors identified so far are integrated into higher-tier risk assessment procedures. When working on standardization of such tests, modern statistical methods should be fully utilized to limit their costs (by using, e.g., fractional factorial designs). Also, there is still a room for better use of ecological knowledge, such as elasticity analysis, to further decrease the complexity of tests on interactions.

Trait based risk assessment

A trait is a phenotypic or ecological character of an organism and describe the physical characteristics, ecological niche and functional role of a species within the ecosystem. Traits-based assessment can be used as a tool for predicting sensitivity of species and also vulnerability of populations based on their characteristics.

Traits-based bioassessment has a long tradition of using traits to explain or predict variation in ecological system condition, (Usseglio Polatera et al., 2000; Poff et al. 2006;). Whereas taxonomy can be regarded as a higher-level expression of the genetic composition of organisms, traits can be seen as their functional consequence (Van den Brink, 2008). The hypothesis that species traits determine their vulnerability to certain types of stressor can be used in two ways in bioassessment. Firstly the absence of certain species that contain certan traits at a particular site can be used to diagnose the stressor reponsible for the impairment (e.g. Culp et al., 2011; Horrigan and Baird, 2008). The basis of this traits-based aquatic bioassessment is that changes in the environmental and biological conditions of a water body can alter communities of e.g. plankton, invertebrates and fish in a predictable way through changes in the relative abundance of tolerant and vulnerable taxa (Thienemann 1918). Therefore, the structural composition of a community can give an indication of the intensity of impact due to different factors or stressors on that community (Kolkwitz and Marson 1902; Wiederholm 1980; Peeters and Gardeniers 1994). Consequently, species abundances that can be correlated with the intensity of a specific stressor can be used as explicit indicators of impacts. The indicator value of any such species is a function of their biological and ecological characteristics, specifically, their traits (e.g. Usseglio-Polatera et al. 2000). Secondly the absence of certain species can also be used as a diagnosis for the stressor by using trait data bases to predict their vulnerability to the stressor. An example of this approach is the SPEAR index (the Species At Risk concept; Liess and von der Ohe 2005). They combined pesticide sensitivity and recovery related traits of species to predict and identify the effect of pesticides on aquatic stream communities. The identification of trait combinations that were associated with a specific environmental parameter (stressor) enabled the effects of this parameter to be identified on the trait composition of the community, even though a multitude of other parameters were affecting the composition of taxa within the community.

The hypothesis is that the vulnerability of species to a stressor is a function of their biological traits such as morphology, life history, physiology and feeding ecology (Baird and van den Brink, 2007). Lately, some work has been done on the relationships between (invertebrate) species vulnerability and chemical stress (Van den Brink et al., 2011a). In their review, Rubach et al. (2011) hypothesise about linkages of traits to processes that affect population vulnerability in ecotoxicology, i.e. being defined by the vulnerability factors external exposure, intrinsic sensitivity, demography and recolonisation. Van den Brink (2008) uses a similar approach, but defines vulnerability as a combination of intrinsic sensitivity, recovery potential and ecosystem interactions. Though traits-based assessment is a promising concept and is used in modern ecotoxicology, a few studies are available in literature on its practical applicability, although first results looks promising (e.g. Rubach et al., 2011).

For predicting sensitivity of species the hypothesis is that the sensititivity of species to a toxicant is a function of their biological traits such as morphology, physiology and feeding ecology (Baird and van den Brink, 2007). Lately, some work has been done on the relationships between (invertebrate) species sensitivity and chemical stress (Rubach et al., 2012; Van den Brink et al., 2011a). Using available data bases, Rubach et al. (2010) and Ippolito et al. (2012) aimed to explain the sensitivity of several freshwater macroinvertebrates toward different classes of plant protection products: organophosphates, carbamates and pyrethroids, but concluded that EC50 might not be the best representative for sensitivity. Results of dedicated experiments performed by Rubach et al. (2012) showed that the toxicokinetics of chlorpyrifos in aquatic invertebrate species is influenced by traits related to size, lipid content and mode of respiration of the species. For predicting vulnerability of populations – for example against agricultural pesticides – it was identified that a toxicant pulse is related to biological traits such as sensitivivity, life cycle characteristics and recolonisation ability (Van den Brink, 2008; Liess and von der Ohe 2005). The combinations of these traits have been used in field monitoring investigations to link population sensitivity to the magnitude of contamination (Liess and von der Ohe 2005; Schäfer et al. 2007; Burgert et al 2011).

In conclusion, a more functional description of ecosystem structure and function can be obtained if communities are expressed as combinations of trait characteristics rather than combinations of species. Since trait composition is governed by environmental conditions, traits have predictive potential for species responses to environmental stress (Van den Brink et al., 2011a). Traits also have the advantage that they add a mechanistic description of the relationship between toxicants and species to the risk assessment (Rubach et al., 2010). Therefore traits have a great potential for use in prospective (Rubach et al., 2011) as retrospective (Culp et al., 2011; Liess and Von der Ohe, 2005) risk assessments. Before their mainstream use in risk assessment, however, many problems have to be overcome (Van den Brink, 2011b), with maybe the development of specific traits approaches for specific (chemical) stressors as the biggest challenge (Ippolito et al., 2012).

One of the bottle-necks for the development and application of the approach is the lack of data for the precise characterisation of suitable traits (Van den Brink et al., 2011b). This is particularly relevant in the cases requiring particular traits describing detailed anatomic characteristics as well as physiologic or metabolic patterns (e.g. describing sensitivity of chemicals with specific toxicological mode of action).

The use of advanced statistical methods for data treatment, such as statistical approaches currently used in chemometrics, may also improve the power of the approach (Baird et al., 2011; Ippolito et al., 2012).

Improving the scientific bases for the development of extrapolation approaches

The final goal of ecological effect assessment is estimating levels of chemicals (e.g. a PNEC) that should be assumed as safe for exposed ecosystems. All the issues described above may provide tools suitable for a more ecologically sound assessment of safe

concentrations. However, a PNEC should take into account all uncertainties inherent with experimental data. Therefore, in most cases, extrapolation procedures must be applied (Solomon et al., 2008).

The current procedures for PNEC calculation are based on the application of assessment factors (AF) which are a function of the quality and quantity of information available (EC, 2003). Sometimes, precise rules are defined for the use of AFs. This is the case of the deterministic procedure based on the application of AFs (usually from 10 to 1000 as a function of data availability) to the base set of ecotoxicological data. In other cases, as for the use of the SSD approach or mesocosms data, a range of AFs (e.g. from 1 to 5) is proposed to be evaluated case by case on the basis of an expert judgment (e.g. Brock et al., 2006).

Even if the selection of the proper AF is roughly based on a general ecotoxicological experience, the science behind it is not clearly defined and a large margin of arbitrariness and non science-based pragmatism exists. Moreover, in the case of SSD, the arbitrariness and subjectivity is increased by the lack of defined rules (i.e. when to apply which factor). The concept of expert judgment is fundamental in any assessment based on procedures that are more complex than a few simple measures producing objective numbers. However, for regulatory purposes, the transparency of the judgment is essential (e.g. Maltby et al., 2005). This is not always the case in existing regulatory exercises.

The increasing complexity of risk assessment procedures that may occur if more ecologically-based approaches, such as those proposed in the previous chapters, will be applied, would make this problem even more relevant.

There is a clear need for developing tools suitable for a more scientifically sound assessment of extrapolation procedures based on calculated estimates of uncertainties, whenever possible based on statistical evaluations.

Ecological modelling

Most of the issues described above should be based on the knowledge of a number of ecological patterns such as population dynamics, trophic interactions, energy balance, etc. The description of these complex patterns for the huge variety of aquatic and terrestrial ecosystems under normal and stressed conditions cannot realistically be achieved through experimental data. Like in current exposure assessments, also in the effects assessment there is therefore a need for suitable modelling approaches capable to describe and predict changes in ecosystem structure and functioning under changing environmental factors.

Ecological modelling has significantly improved in the last few years. However, a challenge for a practical application in risk assessment is the selection and the development of relatively simple tools, capable to produce the information in a form which is useful in a general regulatory context.

Here, we focus on dynamic models of ecological systems, which represent processes and thus causal relationships. This is in contrast to statistical models (also referred to as descriptive or empirical models). Dynamic models are simplified representations of real systems that are designed to answer certain questions. They are used to explore the consequences of our assumptions about how the real systems work (Grimm 2010).

Models can be designed to contribute to general understanding. Such models are usually quite simple, which implies numerous simplifying assumptions. Often, such models are too generic to be related to real data or to make specific, testable predictions. However, ecological models are also increasingly used to support decision making, including risk assessment of chemicals (Schmolke et al. 2010a, b; Galic et al., 2010). In such cases, models are usually more complex because they need to be linked to data and observations. The key question, then, is whether the model captures the internal

organization of the real system good enough to base decisions on its predictions (Rykiel 1996).

Nowadays, virtually all applied ecological models allows to take into account spatial and temporal heterogeneities, stochasticity, and the representation of large numbers of different entities and processes. The key challenge is to make applied models complex enough to be sufficiently realistic, but at the same time simple enough to be understood. Moreover, tying models too closely to data by "overfitting" them can impair their flexibility, in particular when it comes to predict responses to new environmental conditions, or to use the model for different regions. On the other hand, if the link to data is too weak, it can be hard to convince decision makers that the model is realistic enough for its intended purpose. In any case, understanding model behaviour and output is mandatory also for applied models. Otherwise, models would be "black boxes" which were to be trusted blindly; obviously, this would not be a good basis for decision making.

A wide range of types of dynamic models exist in ecology. In the following, a short overview of the main types is given (see also Thorbek et al. 2010). It should be noted that in ecotoxicology, ecological models are grouped with individual-level effect models such as TK/TD and DEB models in the category "mechanistic effect models".

Differential/difference equation models

Ordinary differential equations (ODE) are continuous in time and include a desired function (also called the "solution" of the equation), for example population size over time, and its first and sometimes higher derivatives (Otto and Day 2007). ODEs are the basic tool of any theoretical science. Single or small sets of ODE are not used very often in applied ecological models, but they still are very useful for simpler systems in ecotoxicology, in particular for TK/TD models (Jager et al. 2011). Another example of the use of differential equation models in ecological risk assessment is described by De Laender et al. (2008). If ODEs are used for ecological systems, then usually in larger sets of equations, each equation describing, e.g., a cohort, a species, or a compartment. Still, ODEs are limited in representing spatial effects, local interactions, and life cycles of individual organisms.

In ODEs, infinitesimally small time steps are considered so that formal methods of calculus apply (Otto and Day 2007). For quite a few problems, difference equations are used instead, where time proceeds in steps of a fixed length, which could be hours, days, or years. Such models can directly be simulated on computers, which by their architecture proceed in discrete time steps as well. Modellers need, however, to take care that it is sufficient to summarize all the small changes of, e.g., population size within a year in a yearly net change. Note that matrix models are essentially linear difference equation models.

Matrix models

Matrix models are sets of linear difference equations that describe the dynamics of age- or stage-structured populations. Each equation describes, proceeding in discrete time steps of, for example, years, how the number of individuals in a certain class of the population changes. The parameters of these equations are the class-specific vital rates, i.e. mortality and fecundity. The parameters of all equations build a matrix which can be analyzed using methods from linear algebra. For example, the smallest eigenvalue of this matrix yields the population growth rate.

Matrix models are mainly used for projections of, for example, a population's growth rate (Stark 2010, Caswell 2001).

The advantages of matrix models include: their clear structure which makes communication simple; data requirements are clear; population growth rates can easily be calculated; the sensitivity of different age classes to changes in their parameters can

easily be checked. The elements of the matrix represent average values observed under certain conditions. Matrix models project the consequences of these conditions into the future. For example, we can ask: what if every year 4% of the second and third age class are lost due to pesticide application? Does the population still have a positive growth rate?

The disadvantage of matrix models lies in the assumption of constant demographic rates, which is unrealistic. Consequently, projected and realized growth rate are not the same, and often it is not clear how projected growth rate can be used to assess the risk of "adverse effects". Matrix models are thus mainly a tool for obtaining basic understanding, for comparing species with different traits, and for initial screening of the effects of a certain chemical, or other stressors, corresponding to tier 1 in current risk assessment schemes.

Dynamic Energy-Budget models

These are individual-level, not ecological models, but they are increasingly used as submodels in population models. DEB theory (Kooijman 2010) provides a quantitative framework for modelling the acquisition and use of resources for organisms over the entire life cycle.. The theory assumes that the mechanisms which govern metabolic organization are similar among species. Therefore, the same basic model structure can be used for, in principle, all animal species. DEB models have been successfully used for making predictions at the individual level in a range of disciplines including ecotoxicology, aquaculture, and ecological stoichiometry (Nisbet et al. 2000, Kooijman 2010, Van der Meer 2006). Combining DEB theory and individual/agent-based models (ABMs, see below) is promising as it would combine the flexibility of ABMs with an individual-level model that is based on "first principles". For chemical risk assessment, DEB models are particularly relevant and interesting because the effects of chemical stressors on DEB parameters can be assessed using laboratory data.

Individual- and agent-based models

Individual- and agent-based models (IBMs and ABMs) are simulation models that describe individual organisms as discrete and unique entities (Grimm and Railsback 2005). Population-level dynamics and patterns emerge from the interaction of the organisms with each other and their biotic and abiotic environment. IBMs and ABMs are analysed by performing controlled simulation experiments (Peck 2004). They evolved along different pathways, but the differences between them are fading away. Therefore, we will refer to them as ABM, or agent-based models, in general. It is important to note that, depending on the purpose of the model and the data and time resources available for their development, ABMs can be quite simple (Grimm et al. 2003; Van den Brink 2007), whereas others can be quite complex and require years for development, testing and parameterization (Railsback and Harvey 2002, Stilman and Goss-Custard,2010).

ABM describe the life cycle of individual (discrete) organisms. The entities of an ABM – individuals/agents, habitat units, and the abiotic environment – are characterized by sets of state variables and attributes, e.g., sex, age, body mass, position, type of behavior (individuals), vegetation cover, soil moisture, food level (habitat units), or temperature, rainfall, disturbance rate (environment).

Since ABMs are implemented as computer programs, not as sets of equations, they were notoriously hard to communicate and, in turn, understand. Recently, however, a common protocol for describing IBMs was proposed (Grimm et al. 2006) and is increasingly used (Grimm et al. 2010), the ODD protocol (Overview, Design concepts,Detail). ODD provides a common structure for ABM descriptions, but also helps designing ABMs in a structured way.

ABMs can predict the response of population to unprecedented conditions if they were designed for this purpose. Examples include trout models of Railsback and co-workers (Railsback and Harvey 2002, Railsback et al. 2005), shorebird models of Goss-Custard, Stillman, and co-workers (Stilman and Goss-Custard,2010,), and recent applications of the ALMaSS framework for ABMs (Topping et al. 2009). All these are "full-fledged" ABMs as they are driven by "first principles", e.g. habitat choice, foraging, or physiology; consequently, demographic rates are not longer imposed but emerge from the organisms' behavioural or physiological response to changes in their environment.

The disadvantages of ABMs are linked to their complexity: developing and testing ABMs can be very time consuming and requiring vast amounts of data and empirical knowledge. With the trout, shorebird, and ALMaSS models mentioned above it took years until they could be used and validated for the first time (subsequent applications require much less time, though). Setting up such "full-fledged" IBMs should be compared to setting up a virtual laboratory, which is also time-consuming and expensive, but once it is there, all kinds of simulation experiments (Peck 2004) can be performed.

State of the science

In the academic literature, more than 200 publications address questions related to the effects of chemical stressors at the population level. Galic et al. (2010) reviewed 148 publications and identified five application areas in which ecological risk assessment could benefit from using ecological models:

1. Relevance of effects observed on individuals for the population level
2. Extrapolation of effects of a tested exposure pattern to other, untested, exposure patterns
3. Extrapolating recovery processes, from individual to population level recovery, including recolonization
4. Analysis and prediction of possible indirect effects in communities
5. Bioaccumulation and biomagnification within food chains or food webs.

All five areas are represented in the literature, but the focus is on extrapolation form individual-level effects to populations. Galic et al. conclude, however, that the lack of "clarity of protection goals in legislative documents" makes it hard to directly link the reviewed literature to regulatory risk assessment. In a review with similar scope, Schmolke et al. (2010a) arrived at the same conclusion and emphasized that in the academic literature models are usually not sufficiently well communicated, analyzed, and related to endpoints relevant for risk assessment.

Thus, although virtually all recent publications on the future role of ecological models for ecological risk assessment agree that they are badly needed above (Forbes et al. 2009, 2010, 2011; Thorbek et al. 2010), currently the gaps between academic practice and requirements for regulatory decisions, and between the potential of ecological models and vague population-level protection goals are too large.

Future development

Ecological models are, in fact, already successfully used in environmental decision making in other fields. A recent example is wildlife epidemiology. If diseases of wildlife impose high risk on human health, as with rabies, or on farm animals, as with classical swine fever, European-wide initiatives are taken to eradicate them or to have strategies for confining outbreaks. Thulke and co-workers (e.g., Eisinger and Thulke 2008, Thulke et al. 2011) have been developing ecological models of relevant wildlife populations, e.g. red fox or wild boars, for more than 10 years. Since about 2008, their rabies and classical swine fever models are used as the basis of EU regulations and directives.

Thulke and Grimm (2010) describe how decision makers slowly but surely were convinced that decisions based on these models would make management more efficient.

Several factors made this possible. The rabies model, for example, made a suite of correct independent predictions, i.e. predictions of features of the real system that were not used for model development and calibration. The models could thus be shown to be "structurally realistic", i.e. capturing the most important factors regarding the questions asked. This realism was achieved by basing model design and testing on multiple patterns observed in the real system ("pattern-oriented modelling", Grimm et al. 2005, Grimm and Railsback, 2012). The models were also of only moderate complexity, easy to communicate, and fully understandable via controlled simulation experiments.

Most importantly, however, the models could directly be shown to lead to better decisions than the "mind models" on which management directives were based so far. For example, "ring vaccination", which was successfully used for other epidemics, was favoured by decision makers also for confining outbreaks of rabies, but the rabies/red fox model clearly showed, that in this case it was much more risky than a "compact" vaccination strategy, were a circular area around the first reported occurrences is vaccinated.

Further examples of models that are used for decision making are the predictive ABMs mentioned above, the trout model of Railsback and Harvey (2002) and the shorebird models of Goss-Custard, Stillman an others (Stillman and Goss-Custard 2010). In forest management, the individual-based model SILVA is already used in Bavaria, Germany, as the basis for detailed economic planning of regional forest authorities (Pretzsch et al. 2002). The tropical forest simulator FORMIND (Huth et al. 2005) is used to develop strategies for sustainable management of tropical rain forests in Asia, Africa, and South America. In marine fishery, matrix models are used to support decision making; the correponding data are based on harvest data and quite complex "fishery-independent" surveys (Terceiro 2005). A common feature of all these models is that they were developed over at least 5 years; they are well-tested and documented, structurally realistic; finally and perhaps most importantly, they are, despite their complexity in terms of model structure and processes, conceptually simple enough to be assessed by decision makers.

Potential use in risk assessment

The pros have been described repeatedly in various publications (Forbes et al. 2009, 2010, 2011, Thorbek et al. 2010) and can be summarized as: If we want to make risk assessment of chemicals more ecologically relevant, ecological models are the only way to go. Experiments in mesocosms and the field will continue being important, but they are too limited to cope with the complexity, extent, and time scale of ecological systems. The cons are the current gap between academic practice and the requirements for regulatory risk assessment. Models need to be better communicated, validated, and tied to endpoints relevant for risk assessment. On the other hand, a clearly defined suite of relevant species, scenarios, and protection goals needs to be developed to make better use of the potential of models.

There are three main barriers to substantial progress: (1) the lack of guidance for Good Modelling Practice that transfers academic modelling practice into a framework that makes model results more useful for regulatory decision making; (2) the lack of well-tested and –documented example models of relevant species which can be used to define environmental scenarios, to explore the added value of models for risk assessment, and to define appropriate population-level endoints; (3) researchers trained in both ecological modelling and chemical risk assessment.

Currently, the EC-funded Marie Curie Initial Training Network "CREAM" (September 2009-August 2013; http://cream-itn.eu) is addressing these three issues: guidance for Good Modelling Practice is developed, referred to as "TRACE" (Transparent and Comprehensive Ecological Modelling Documentation; Schmolke et al. 2010b); in more than 15 case studies specific models are developed; and 23 early-stage researchers are trained.

In conclusion, to make risk assessment of chemicals ecologically more relevant and link it better to the protection of ecological systems and the services they provide, there is no alternative to using ecological models in the future.

In the short term, specific models exist which can, and partly have already been, used for regulatory risk assessments, such as the individual-based model of aquatic invertebrates, MASTEP (van den Brink et al., 2007), or agent-based models of certain vertebrate species developed within the ALMaSS framework (Topping et al., 2009). However, currently each model needs to be evaluated by decision makers on a case-by-case basis, which is inefficient and goes beyond the resources of most European regulatory authorities.

In the medium term, the CREAM project is likely to have a major impact on the attitude of industry and regulators towards ecological models. Moreover, CREAM will deliver guidance for Good Modelling Practice, i.e. TRACE. Currently, the EFSA panel on plant protection products is discussing the development of guidance for Good Modelling Practice. There, TRACE is likely to be taken into account. Furthermore, a SETAC workshop is planned where experience with existing models of small mammals and aquatic invertebrates will be used to explore jointly and agree on relevant scenarios, endpoints, and protection goals. All this means that, ecological models will increasingly be used for risk assessment in the medium-term.

In the long-term, industry and regulators hope that standardized models and scenarios similar to the FOCUS models, and scenarios for environmental fate models will be used. Indeed, an EU-wide harmonized process similar to FOCUS would be the most efficient way to establish the use of ecological models as an integral and routine part of ecological risk assessment of chemicals at the European level as fast as possible. FOCUS needed about 10 years to be put into practice, and the time frame for ecological models seems to be, based on lessons from existing decision support models, similar. However, there are important differences to fate models and FOCUS. Ecological systems are more complex than the physical environment, therefore it could be dangerous to aim for standard models, which tend to be used uncritically and may prevent development and further monitoring and experiments. Therefore, a process similar to FOCUS for ecological models should aim for a standardized framework of protection goals, endpoints, relevant species and scenarios, assessment criteria for models, Good Modelling Practice, and small set of standard design frameworks for ecological models. Matrix models are such a design framework, and developing similar frameworks for agent-based models certainly is possible. They should be transparent, open to be used by any modeller, and routinely be scrutinized, updated, and improved.

4.4.Ecological risk characterization

4.4.1. Limitations and drawbacks of current procedures

Current procedures for risk characterisation are based on the comparison between an environmental concentration (usually a PEC) and an indicator of the effects likely to occur. Usually, the indicator of effects is a PNEC, assumed to be a concentration of the chemical that does not affect ecosystems. In other cases (e.g. according to the pesticide directive), it is a more specific ecotoxicological endpoint. A PNEC is usually derived on the basis of a few ecotoxicological data from single species testing, even if, recently, more complex approaches (SSD, mesocosms) are frequently used.

Risk characterisation is represented by a numeric value (PEC/PNEC ratio or TER) that represents a threshold for the protection of ecosystems or of parts of them.

The pragmatic value of this approach, particularly for regulatory purposes, is clear. It allows a quantitative expression of the risk with a relatively small amount of information by applying a simple and transparent procedure. Moreover, on the basis of the present ecotoxicological knowledge, it can be assumed as reasonably protective (sometimes even

overprotective). Therefore, it represents a fundamental starting point for chemical regulations. However, in spite of these advantages, the actual consequences on the health of natural ecosystems produced by a PEC/PNEC higher than 1 are unknown.

Moreover, traditional procedures are generally applied to standard scenarios at different scales (local, regional, continental) assumed as representative of an ideal European environment. In some cases, a few environmental scenarios (e.g. FOCUS) are assumed as representative of the huge variability of European situations. It follows that current procedures are not adequate for site specific ecological risk assessment.

In the following schemes, the traditional risk characterisation approach is compared with a more complex approach capable to account for some issues related to ecosystem complexity.

In the previous chapters, some relevant issues, capable to improve risk assessment approaches and to increase our understanding of the behaviour of ecosystems under stress have been listed and synthetically described. The current status of their applicability for regulatory risk assessment is discussed below.

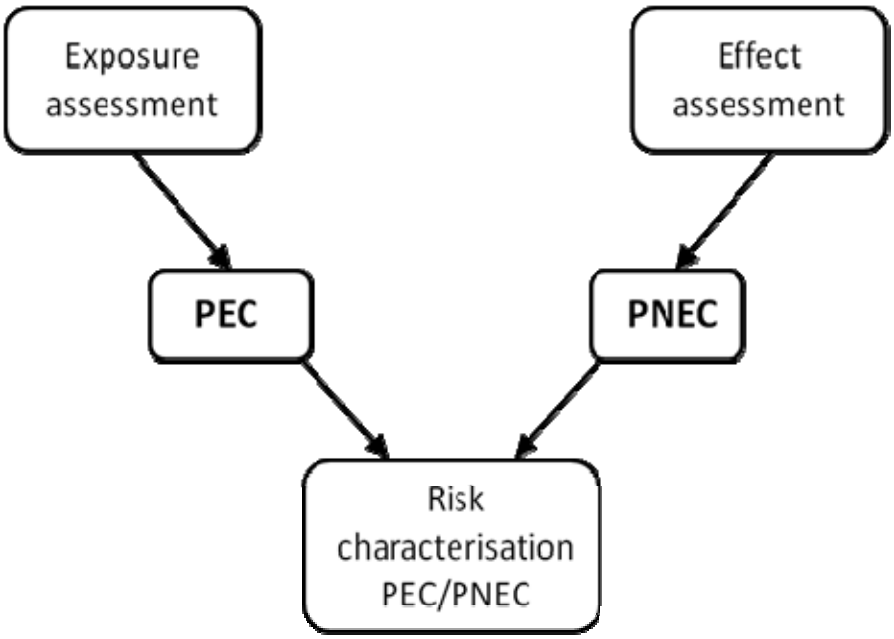


Figure 2: The traditional approach

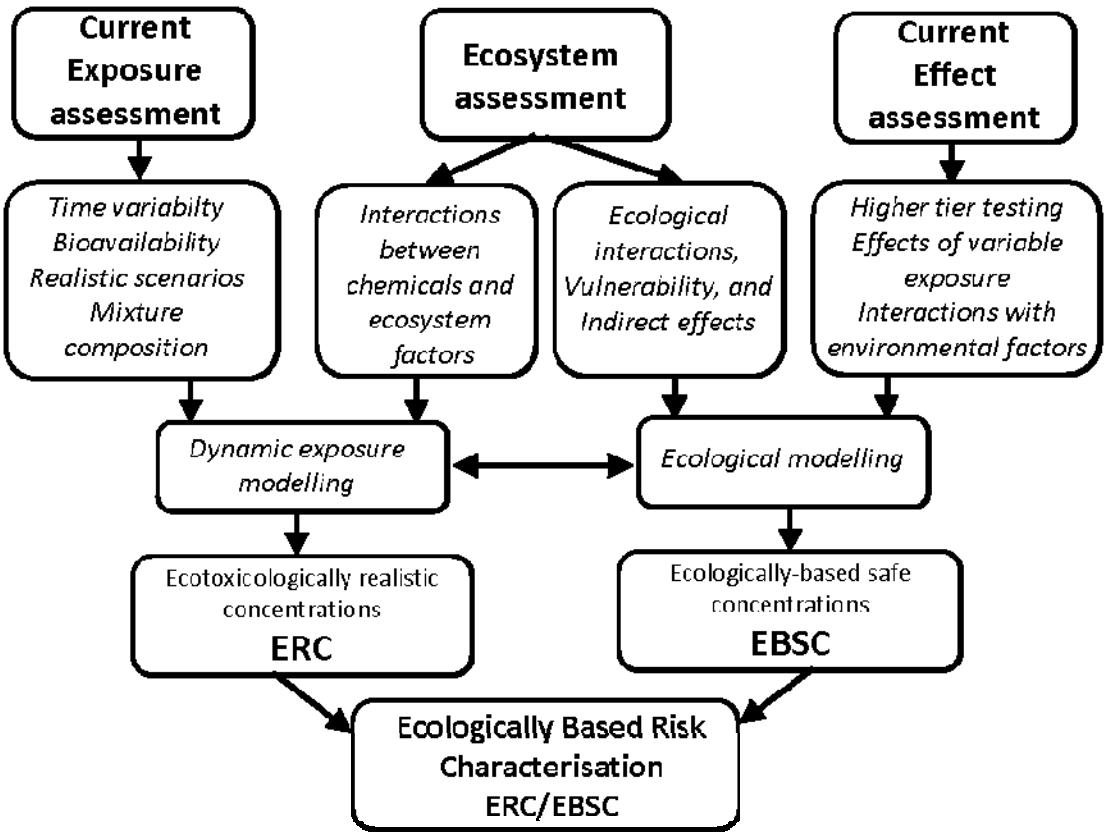


Figure 3: A more ecologically-based approach

4.4.2. Applicability of current procedures for risk characterisation

Requirements

The ecologically-based scheme for ecological risk characterisation proposed above may represent a substantial change from the traditional procedures currently applied. It is based on a number of scientific issues that may contribute to a better understanding of the complexity of ecosystems and of the interactions between ecosystem factors (biotic and abiotic) and stressors.

There is no doubt that these issues are of essential scientific relevance and that addressing them would bring more ecological realism to ecological risk assessment. However, the question of their practical applicability, particularly for regulatory purposes, remains open.

In order to be used in a regulatory context, new scientific approaches must fulfil some important requirements:

- their scientific soundness must be largely recognised and accepted by the international scientific community;
- they should be specific for different types of stressors;
- they must be applicable with (relatively) simple tools that should reach a certain level of methodological standardisation;
- they should produce quantitative results in order to allow the development of triggers, benchmark levels, rankings, etc.;
- their applicability and the interpretation of the results must be transparent and relatively objective; as far as possible the use of a subjective "expert judgement" should be avoided;

- the results should be replicable and reproducible with acceptable approximation.

Therefore, it is important to answer a number of questions that may clarify the potential usefulness of the described approaches in a short or long time perspective. In synthesis, some of these questions may be the following:

- Are the issues described above realistically applicable for risk characterisation?
- Do they respond to the requirements of European regulations?
- Are they ready to be used?
- Is the science behind them sound enough?
- Which are the needs and priorities for future research?

Exposure assessment

While in exposure assessment a number of tools are available to predict the fate of many non-polar organic chemicals in several compartments, many uncertainties and lack of proper tools still exist at different levels in exposure assessment:

- **Assessing sorption and bioavailability**: while tools are available for non polar organic chemicals and metals, a consistent lack of knowledge exists for polar and ionized chemicals, currently making the predictions nearly impossible.
- **Nanomaterials**: very little is known to characterize fate and exposure. However research (including EU projects) is ongoing
- **Better use of monitoring data**: need for criteria and protocols for obtaining and comparing monitoring data in RA, especially for evaluating the fate of chemical mixtures, including metabolites. Also data at short temporal resolution (e.g. hours) would be required for developing/calibrating predictive approaches. There is a need to collect and report monitoring data to explore spatial and temporal variability of concentrations and to assemble them in databases according to QA/QC criteria to be employed for model development, verification and validation.
- **Need to verify and harmonize the physical-chemical data** obtained in the REACH regulation according internationally recognized data quality requirements
- **Improvement of modelling approaches:**;
    - Models should be developed to be capable to handle polar and ionized chemicals as well as nanomaterials
    - There is a need to develop models capable to predict time and space variable concentrations in order to be compared to monitoring data and account for realistic exposure situations, rather than "generic" environments (to devise the shape of concentration trend at different sites (e.g. various part of a river) in time, (e.g. constant vs. pulse).
    - There is a need to develop realistic scenarios with a variation of environmental characteristics to reflect the ecological variability of conditions in all European geographic areas or ecological conditions.
    - Specific organism parameters should be developed to extend modelling tools capable to understand bioaccumulation of many more representative organisms in aquatic and terrestrial systems;
    - Models should be developed capable to describe the food web pathways of chemicals. For terrestrial systems, this should include the role of vegetation uptake in driving/regulate the input to the food chain and organic carbon cycle.

Effects assessment

As explained in the previous sections, several issues must be included in ecological risk assessment in order to achieve the objective of protection of structure and functions of ecosystems. These issues include some processes that need to be known and some tools that may be useful in ecological risk assessment procedures. Some of them already have sound scientific bases and may be practically applied, even if improvement can still be achieved. In other cases, even if the usefulness of some approaches is clear, the present level of knowledge is still too limited and suitable tools are not sufficiently developed for a practical application in risk assessment. These issues must be considered as relevant priorities for ecotoxicological research in the near future.

- **Higher tier effect assessment.** Mesocosm data and SSD are already used successfully in ERA. Even if several improvement are possible (particularly for the development of more standardised methods capable to produce more reproducible results), they already represent a powerful tool for improving ecological realism of risk assessment. A relevant problem for their use in regulatory assessment is the improvement of transparency in the evaluation of the results, reducing the need for a variable and disputable expert judgement. In particular, a relevant priority for research is the development of statistically-based tools capable to quantitatively assess uncertainties and to improve the transparent use of these approaches (see section 4.1.3.2.10).
- **Effect assessment for complex exposure patterns.** Accounting for time and space variability is a key issue for exposure assessment. For assessing the effects of such variable exposures, toxicokinetic/toxicodynamic (TK/TD) models seem to be the most suitable tool currently available (see section 4.1.3.2.2). The improvement of these models and, in particular, their experimental validation with different groups of living organisms representative of aquatic and terrestrial biological communities is a relevant priority for research.
- **Effect assessment at low hierarchical level**. Research efforts on processes occuring at the sub-individual and molecular level (e.g. gene expression) are rapidly growing (see section 4.1.3.2.3). Omics tools can detect substance-specific effects at the low exposure concentrations prevailing in the environment. However, at present, the relationship between molecular effects and responses at higher hierarchical levels (population, community) is largely unknown. Considering that protecting high hierarchical level is the goal of environmental protection (see section 4.1.3.2.5), the usefulness of molecular approaches in ecological risk assessment remains to be established. Until then, this information and these tools are of limited value for ecological risk assessment..
- **Ecosystem vulnerability**. The relevance of vulnerability evaluations for populations, communities and ecosystems is recognised, particularly for site/region-specific risk assessment. However, to have practical applications in risk assessment, ecosystem vulnerability should be expressed in quantitative terms. This will allow the comparison of different ecological systems the development of quantitative risk characterisation procedures. This is not an easy task, considering that vulnerability is a relatively complex concept. Some tools have been developed for quantifying vulnerability (e.g. SPEAR) while other approaches are preliminary proposals requiring careful calibration and validation (see section 4.1.3.2.4). The development and validation of methods capable to assess vulnerability of aquatic and terrestrial ecosystems to different types of stress factors, represents a priority research need.
- **Indirect ecological effects.** Indirect effects due to ecological interactions are a key issue for assessing effects at the ecosystem level. They can override direct effects and can either mitigate or exacerbate direct effects. A few experiments and community models have demonstrated the potential importance of indirect

effects, showing that they are a key issue for assessing effects at ecosystem level, but overall knowledge for use in risk assessment remains poor. Therefore, indirect effects must be considered as an important research need. More comprehensive studies are needed, based on experiments, inverse statistical modelling and ecological modelling (see section 4.1.3.2.7).

- **Interactions with environmental factors.** While the science behind the assessment of effects of several chemicals in combination may be sound enough for proposing the introduction of mixture risk assessment in international regulations, the knowledge on the interactions of toxic chemicals with other potential stress factors is much less developed. Even if some information is available in the recent literature, studies on the interactions of toxicants with other environmental factors have not received enough focus for ecological risk assessment. Considering the relevance of the issue, it must be considered a priority for future research needs (see section 4.1.3.2.8).
- **Trait based risk assessment.** Trait based approaches (see section 4.1.3.2.9) represent a very promising tool capable to complement taxonomically based assessments with a functionally based assessment. At present, they represent a tool for vulnerability analysis and for many other approached relevant for ecological risk assessment. The development of tools and databases for improving the application of trait based ecological risk assessment represents a priority need for research.
- **Ecological modelling.** Ecological models, often in combination with individual-level effect models like TK/TD or DEB models, are the only way to fully take into account "ecology" in risk assessment. Current modelling practice is too diverse and non-transparent for regulatory risk assessment. However, good modelling practice is currently under development. A number of models can already be used, for specific questions, but currently needs to be assessed by regulators on a case-by-case basis. To make full use of the potential of ecological models, a concerted action is needed to agree on standard scenarios, ecologically relevant test species and endpoints, acceptance criteria of ecological models, and to develop well-tested, flexible models that are both routinely used and improved. (See section 4.1.3.2.10). Ecological modelling, with considerations for its application in the regulatory arena, should be considered as a important research need.

Ecologically based risk characterization

Once the relationship between varying exposure concentrations and consequent effects on populations and communities are clear and scientifically sound, dynamic exposure models and ecological models can be linked to study site specific responses to chemicals or to construct exposure scenarios for a variety of environmental systems in order to be used in the regulatory framework.

5. HUMAN-HEALTH RISK ASSESSMENT

This analysis focusses on the identification of a framework for the future development of the methodology to enable a needed paradigm shift in the assessment of the risks from chemical stressors. The reasons for the need for such a paradigm shift are set out. One of the long-term future challenges would be to include other type of stressors (physical and biological) in the global risk assessment for human health. It is recognised that many of the changes identified will take several decades or more to be fully realised for risk assessment purposes. The analysis does not consider risk management issues including particular regulatory constraints on the acceptability of these changes.

5.1. Aims of the new risk assessment methodologies

The starting point in any examination of the required developments needs to be a vision of what a future risk assessment should aim to achieve. Risk assessment in humans has the great advantage over ecological risk assessment that only one species is involved. However, there are still major challenges to be addressed, including how to characterise the potential high diversity of individuals in response to specific chemicals.

The following objectives and sub-objectives are proposed in identify a future strategy.

The general objective is to achieve a very high level of accuracy in the estimation of all significant adverse effects that may occur in man and/or the environment as a result of external exposure of humans to multiple stressors.

The general objective may be achieved by addressing the following sub-objectives:

- To identify the extent to which it is feasible to achieve greatly reduced reliance on routine tests in experimental animals and those areas where the greatest advances are likely.
- To achieve low cost and rapid testing systems.
- To recognise and apply promising emerging technologies.
- To be sufficiently adaptable and flexible to be applicable to the challenges posed by new technological developments resulting in new industrial processes and products (stressors).
- To enhance the scientific underpinning of each stage of the risk assessment process and to use this to enhance understanding of the causes and confounding factors to disease.
- To increase the transparency in the communication of the risk assessment outcomes, particularly in terms of the scope (e.g. realistic risk estimations versus lower-tier precautionary approaches) and uncertainties, including a proper communication of the expected variability in the risk for different population groups /conditions.

5.2. Towards a new conceptual framework in risk assessment

5.2.1. The need for a new conceptual framework

Although currently used human risk assessment methodology has served us well to date, a number of imperatives demand a complete reappraisal of the methodology itself and of its applications. Risk assessment has an essentially practical purpose. Therefore, it is essential to take account of the future contexts in which risk assessments may be

applied, when identifying a suitable conceptual framework for future risk assessment procedures.

The general methodological approach that has been used to provide the data required by the various regulatory bodies to assess the risk to human health from chemicals and other stressors has remained largely unchanged for several decades. However, the range of chemicals and other stressors for which it has become a regulatory requirement to assess the human health risks arising from potential or actual exposure has continued to expand. It is also evident that the risks posed by a number of products from new technologies (e.g. biological products, manufactured nanomaterials) are unlikely to be adequately assessed using current methodologies alone (SCENIHR 2009)

The need for a reappraisal of current practices is reinforced by the growing political/stakeholder concerns regarding:

- The current central methodology making use of animals for toxicity testing;
- The transparency and objectivity of various aspects of the risk assessment process;
- The high cost of meeting the regulatory requirements maintaining a high level of protection for exposed populations which is a disincentive to the development of potentially useful, innovative products.

A new paradigm for human risk assessment is needed that takes into account these challenges and further improves the reliability of future risk assessments. The proposed paradigm is the development of *'an exposure-driven, flexible, tiered approach, drawing continually on advances in technology and scientific understanding of biology, which meets the needs of stakeholders'.*

5.2.2. A flexible tiered approach

The obvious strategy, to both limit unnecessary animal use and to reduce unnecessary costs, is to develop a flexible tiered risk assessment framework. It will be important in establishing this framework to ensure a high level of public-health protection in light of the fact that zero risk is unachievable. An outline of the approach is provided here; further details are set out at the end of each main section.

Tier 1, conservative estimates of human exposure levels need to be made. The aim is to identify chemical/stressor exposure situations where additional hazard data is unnecessary because exposure levels are too low for an adverse effect on human health to occur. This concept provides the basis for the approach known as *'thresholds of toxicological concern'* (TTC). It needs to be noted that progress in the utilisation of TTC and allied methodologies depends on the further development of exposure methodology along with strengthening of the hazard databases which underpins it. In tier 1, for those stressors for which exposure could be above the thresholds for possible effects, the hazardous properties should be identified in qualitative terms. The ultimate intention should be to assess the hazardous properties, based on *in vitro* and *in silico* methods alone, as well on grouping of chemicals and application of read across. Any available human data such as epidemiology findings should also be considered at this stage.

Tier 2 should be applied to all chemicals/stressors that, based on the very conservative exposure estimates, are above the TTC value or based on structural or specific hazard concerns are considered to need further assessment.

In tier 2, a more realistic assessment of exposure should be performed. If this is not achievable, the very conservative estimation should be retained. The relationship of any hazardous properties identified of concern to exposure levels need to be established in tier 2. In the long term this may be based on *in vitro* and *in silico* data only. However, this fundamental change in the nature of the data sought depends on very major progress in the development of suitable alternative methods. If the hazardous properties

are identified as only occurring at unrealistic exposure levels or for other reasons are of very limited concern, then no further assessment may be needed.

Tier 3 consists of an in-depth risk assessment, which will typically involve a range of carefully selected *in vivo* tests. Tier 3 should include consideration of human variability both in the levels, duration and routes of exposure and in the vulnerability to the chemicals effects in other words, the extent and causes of individual variations in susceptibility to the exposure need to be adequaltely characterised. Common practice at present is to assess the risks to population groups from typical (or an arbitrary higher percentile) exposures to a particular source of an individual chemical/stressor. Allowance for the effects on more susceptible subjects is then made by using arbitrary standard default factors.

The recent introduction of the concepts of exposome (i.e. an individuals' lifetime exposure to chemicals) and toxome/toxosome (i.e. the effects in an individual from lifetime exposure to chemicals) draws attention to the need to identify the range of exposure of members of a population group not simply to a specified single source of a chemical but to all sources of the specific chemical (i.e. cumulative exposure). In addition to address the identification of possible effects coming from the simultaneous exposure to related different chemicals, as well as to chemicals and other stressors (i.e. aggregated exposure), as described in the Scientific Committees' previous opinion on risk assesment of chemical mixtures, tier 3 should include the identification of the plausibility of concurrent exposure as well as a detailed mode of action of chemicals, providing the plausibility of independent action, addition and/or interaction.

The tiered approach outlined above is critically dependent on methodological advances. It should not be seen simply as a means of reducing animal testing, but rather as a progressive process, each aspect of which will demand proper validation. In considering the application of new methodologies to risk assessments in the future, their potential contribution to this tiered system should be evaluated.

- Advances in exposure assessment methodology;
- Development of *in vitro* preparations that are representative of the corresponding *in vivo* situations over the long term;
- Establishment of predictive tools e.g. SAR
- Improved knowledge of modes of action;
- Better understanding of individual variability in response to chemical exposures.

Science continues to advance and it is essential that the risk assessment process keeps pace with these developments. New technologies and their potential to be incorporated in the risk assessment process is the central theme of this discussion paper.

5.3.New concepts

5.3.1. Threshold of toxicological concern (TTC)

Introduction and current use

The TTC approach, originally developed to address food contaminants, is designed as substitute for substance-specific information in situations where there is limited or no information on the toxicity of a chemical. It should not be used for any chemical where there is useful hazard data. It is therefore a potentially valuable tool that prevents both unnecessary use of animals and unnecessary in depth risk assessments. The TTC approach is based on the concept that below certain human exposure levels there is a very low probability that a chemical will cause adverse effects to human health

(Cheeseman et al., 1999). These presumably "safe" levels of exposure are derived from extensive databases on the levels of exposure causing various types of adverse effect in humans.

To support the use of TTC, databases of carcinogenicity data from animal studies (Carcinogen Potency Database, CPDB) and other toxicological endpoints (Munro et al., 2008) are available. Both are based on systemic effects after oral exposure. Chemicals with complex chemical structures, however, are not adequately represented in these databases.

The limitations of the current databases mean that, for the time being, TTC should not be applied to the following until substantial, relevant additions to the databases are made:

- Aflatoxin-like, azoxy- and N-nitroso-compounds due to their high carcinogenic potency;
- Metals, organometallic and other inorganic compounds;
- Compounds likely to accumulate substantially in the body, such as polyhalogenated chemicals and ochratoxin A;
- Potent hormones, such as steroids;
- High molecular weight chemicals, such as polymers;
- Proteins (NB due to potential for allergenicity or a range of other biological activities);
- Substances in particulate form. This includes insoluble nanomaterials, in either natural or engineered form.

It should be noted that this database depends solely on findings in animal tests. Current applications in the EU are confined to flavourings. However this is likely to change in the near future in view of the recent EFSA opinion.

Future potential

Consideration needs to be given to how the requisite data gaps might be filled using future methodologies (see section on data bases). In the near future the application of TTC needs to be supported by a greatly improved data base on the properties of existing chemicals and by the application of SAR. In the longer term a data base on modes of action should also inform the use of TTC (Escher et al., 2010).

The use of TTC depends on realistic estimates of human exposure including that of the most exposed population groups. Failing these very conservative estimates of total exposure to a chemical should be used. In the future a data base(s) of exposure information should be available to aid the identification of an appropriate exposure level to be used for the TTC. It should also be used to identify likely exposure to closely related chemicals. Other important needed developments are validated tools for route to route extrapolation or the development of an internal TTC (as the minimally required blood concentrations to show any toxicity).

5.3.2. Tox-21

The current discussion paper should draw on work already carried out by other bodies that have considered what future risk assessments of chemicals might look like. The most important of these is the US National Academy of Sciences publication on '*Toxicity testing in the 21st century: a vision and a strategy*' (Kavlock et al., 2012), (Kleinstreuer et al., 2011), (Dix et al., 2007). This report is concerned primarily with human health risk assessment from exposure to individual chemicals and proposes the following approach for risk assessment:

- Initial examination of the physicochemical properties and prediction of environmental and biological fate of individual compounds. The fate to include possible environmental concentrations, likely metabolites and breakdown products, initial interactions of compounds and metabolites with cellular components and possible toxicities.
- Toxicity testing which comprises two components: toxicity pathway assays in which initial perturbations of relevant biological systems are assessed followed by complementary targeted testing aimed particularly at clarifying uncertainties in the interpretation of the toxicity pathway data.
- Dose-response relationships. This involves three elements of extrapolation:
    - a quantitative mechanistic understanding of the relevant pathways
    - physiologically based exposure modelling
    - utilisation of any suitable human data
- Exposure data based on human surveillance data using biomarkers.
- Since exposure is used only to define realistic doses for toxicity testing, the proposed approach is more of a priority-setting tool rather than an actual risk-assessment approach. It is emphasised that the above framework will take time to develop and that a vital additional element is regulatory acceptance.

Of particular note is the shift from the present paradigm which generally focuses on hazard characterisation to an exposure-driven approach. However, the NAS report does not consider in depht exposure assessment, although this is often the weakest point in risk assessments. It is the view of the Scientific Committees that it is vital that advances in exposure assessment are given priority in the practical development of the new paradigm.

The NAS report places heavy reliance on the use of *in vitro* tests to characterise mechanisms of toxicity (pathways) as a means of focussing further hazard assessment on supplementing information derived from the pathway assessment. It is envisaged that the mechanisms evaluation will be derived primarily through information obtained using genomics, proteomics and metabolomics on *in vitro* systems. It will benefit greatly from research in many fields of biology that are not currently sufficiently considered in building toxicological paradigms. It is advocated that where possible the *in vitro* systems are of human origin. *In vivo* testing is viewed as necessary in the short to medium term to sophisticate the assessment although it is considered that in the long term use of experimental animals may become unnecessary.

In terms of what is required from a methodological perspective to meet this vision the following are prerequisites:

- A wide range of *in vitro* test systems derived from human tissues. The assumption here is that cells derived from humans must be more representative of man *in vivo* than other *in vitro* systems.
- High throughput systems to detect biological changes caused by exposure to the chemical under investigation and enable their interpretation in terms of modes/mechanisms of action.
- Rapid data processing systems to deal with the large amount of data generated.
- Improvements in exposure assessment modelling using information from a range of sources to estimate more realistic exposure levels to be used in in vitro testing.
- TK-modelling to translate effect concentrations in the *in vitro* assays to expected tissue concentrations in humans.
- *In vivo* models to address specific questions to narrow uncertainties in the risk assessment.

Rather brief consideration is given in the NAS report to the following:

- The importance of defining the question(s) in a manner that enables suitable methodologies to be selected
- The time scale and requirements to move from a toxic endpoint based assessment to a reliable mechanistic one
- The role of techniques other than "omics" in achieving the vision.
- Whether human cell derived *in vitro* test systems are a pre-requisite
- How to establish a suitable database for the storage and utilisation of the information derived using the new test systems.
- Whether *in vitro* tests will actually cover all relevant targets/mechanisms of a toxic response in an intact organism.
- The difficulty in data interpretation, and the cost.

It is important that each of these issues is considered in the present opinion.

The National Academy report has resulted in the US-EPA ToxCast Programme. This programme uses a variety of cellular and biochemical assays to generate response "signatures" of chemicals in these assays. By comparing signatures in the ToxCast assays with *in vivo* toxicities of chemicals already undergoing intensive testing, it is hoped to generate predictive signatures for specific toxicities. In addition, ToxCast tries to base risk assessments for chemicals on the lowest concentrations causing a response (clearly related to an adverse effect) in the many assays and comparing these concentrations with predicted steady-state concentrations of the chemicals in humans for priority setting. If the most sensitive response in the ToxCast assays occurs at exposure levels that are well above the expected steady-state concentrations in humans, this would indicate low priority for more detailed risk assessment (Berg et al., 2011), (Stephens et al., 2012).

5.3.3. Exposome

Recently, the concept of the exposome has been introduced to embrace the lifetime exposure of a human to chemicals from conception to death. It has been proposed to be a critical entity for disease etiology (Wild, 2012). The key element in realizing the objective is to be able to determine accurately lifetime exposure of individuals to different chemicals and how these exposures relate to the development of disease. As the exposure is in a constant flux, it would thus require sequential measurements that span the lifetime of an individual, or alternatively a series of snapshot measures representing key stages in the development. Both bottom up and top down strategies are likely to be needed. The bottom up approach involves the measurements or modeling of exposures in air, soil, water, food, etc., to which an individual is exposed whereas a top down approach requires biological monitoring of individuals. The latter represent both exposure to a chemical stressors as well as important endogenous processes that influence toxicity pathways, e.g. physical activities, endogenous circulating hormones, oxidative strees and ageing. s In addition the exposome includes other non-chemical stressors such as an individual's social, economic and psychological environment as well as climate changes.

It follows that exposomic studies are likely to use a mix of tools to describe exposure and adverse effects. Biomarkers are important to determine exposure, but depending on the biomarker chosen prior knowledge of the half-life is required for a correct study design. Several "omics" techniques are used to describe effects, disease progression and (individual) susceptibility factors. Since exposomics will generate large datasets, good data mining is needed to find statistical associations between exposures, effect of exposures and genetic susceptibility linked with any disorder. Since the overall task is daunting, the initial focus is likely to be on chemicals, known to cause significant adverse

effects at chronic exposure levels that might be experienced in reality. It is however important to consider that the analytical work should be hypothesis-driven.

Characterizing the exposome requires both highly sensitive high throughput analytical procedures as well as longitudinal sampling of biological index material, particularly during critical life stages (e.g. fetal development) and will vary with time, due to different diets and lifestyles. Thus, progress in characterizing the exposome will be determined by advances in exposure estimation methodologies and in the understanding of modes of action.

Tools such as personal monitors and implants will be needed to achieve a reasonable analysis of an individual's exposome. Such devices would be of great help in the practical determination of the exposome.

5.3.4. Toxome (toxosome)

It is anticipated that the identification of the toxome will influence the future structure of hazard assessment of chemicals. However the Toxome based upon *in vitro* systems appears not to be able to reflect the complexity of the organisms and its derangement by a chemical.

While traditional *in vivo* and *in vitro* toxicity testing only provide limited mechanistic testing, the introduction of omics technologies reveals the interactions of chemicals with biochemical pathways . A concern is still how to translate information from the cell level, to organs and subsequently to individual and to distinguish between adaption vs.adversity (Bhattacharya et al., 2011) (Krewski et al., 2009).

In Tox 21, the term ”pathway of Toxicity (PoT)” is defined as a biological pathway that, when sufficiently pertubated by a chemical, can lead to adverse effect outcome *in vitro* or *in vivo*. PoT is “molecularly defined descriptors of pathways of substance interference with biological systems ”, in contrast to mode of action that is based upon cell physiology or affected cell function. PoT may be different in humans and animals. POT is a perturbation of a cellular network rather than a specific effect. it is important to bear in mind that if a compound triggers a PoT it does not necessarily indicate a hazard, but a potential for hazard, and thus may require additional testing .

Several PoT may be influenced by the same chemical and their combination forms the Signature of Toxicity, used to describe the disturbance of a chemical on cellular processes that are involved in the adverse outcome. A single PoT does not necessarily results in an adverse effect, but it is likely that a combination of PoT create it together with pathways of defense. By identifying these toxicity pathways hazards alert can be better identified and additional testing could be the consequence. The analysis will require integrations of many different data from several areas and would require the involvement of bioinformatics. Several PoT has been identified, but mapping the entirety of these pathways (hence the ‘Human Toxome Project’) could be a large-scale effort, perhaps on the order of the Human Genome Projects. Knowledge from systems biology and its integration into toxicology is important tools in the development of the Toxome (Keller et al., 2012).

PoT based approaches together with integrated testing strategies will play an important role in the future systemic toxicity assessment.

NIH-sponsored Human Toxome project similar to the Humane Genome project has been initiated.

5.4.Physico-chemical properties

Current use

Physico-chemical characteristics define the nature of a chemical in a specific form, as produced or as packed for a certain purpose. Physico-chemical characteristics embrace a large set of descriptors for atomic/molecular structure and properties. Characteristics such as the crystalline (also including crystal or molecular symmetry/chirality) or polycrystalline form or the size/size distribution or shape/shape distribution of individual grains, domains, particles, crystals and the form of aggregation in emulsions, aggregates, agglomerates, poly or single crystals are often referred to as structural descriptors, which by the nature of physics can be modified by changing temperature/ temperature cycling and by interaction with other media. Hydrophobicity, solubility, boiling point, ionisation potential etc. on the other hand are physicochemical properties which are directly related to a certain atomic or molecular species in specified environmental conditions (pressure, temperature, etc.). For additional information on physico-chemical characteristics, their use in predictive approaches and needs please see 4.2.6.

Physico-chemical characteristics describe the fundamental interactions of a substance or material with its environment, as measured in well described measurement methods. Thereby physico-chemical characteristics is to a significant extent predictive for the more complex 'behaviour' or 'fate' of a certain substance or material when exposed to individual organisms. Physico-chemical properties suggest interactions and reactive properties of a chemical and, as such, contribute to its toxicologic profile. Knowledge of the physicochemical characteristics of a chemical can contribute significantly to the understanding and prediction of its interaction with cells, tissues and organisms.

Over the years many parameters have been defined, measured or calculated that relate to physico-chemical features of chemicals. Parameters that reflect the ability of a chemical to partition between aqueous and non-aqueous phases (partition ratios) include its hydrophobicity/ lipophilicity, which plays a significant role in determining its partitioning into living tissue and therefore its behaviour in absorption, distribution and excretion processes. Originally, the logarithm of the equilibrium concentration of the compound between the octanol phase and the water phase (LogP; $LogK_{ow}$), was deemed indicative to measure the partitioning between a hydrophobic environment and water. More recently, several new parameters, indicative for more complex uptake processes than basic solubility rules, have been introduced to characterise the likely partitioning of molecules in tissue, including a family of pH-dependent values (LogD), reflecting potential ionisation in aqueous systems at various pHs.

Also structural physico-chemical descriptors affect uptake, distribution and fate of a chemical or material and its interaction / toxicity with an organism. While still a matter of discussion, some structural classes of chemicals have been assigned to similar physico-chemical characteristics (e.g. aromaticity), also different morphologies (crystal structure and grain/particle size/shape distribution) and chirality indicate a different interaction profile. Recently, chiral technology has developed to a point where we are allowed several options in enantiomer resolution and preparation techniques, which in turn offer new avenues for human toxicologists to explore the stereochemical properties of these ubiquitous agents.

A critical need is a widely available comprehensive ('open source') database linking physicochemical properties to effects, facilitating the establishment of more quantitative read across approaches.

Towards the in-depth assessments of low-solubility chemicals in the form of particulates a novel challenge is comprised by the necessity to understand particle – biomolecular (predominantly protein, but also with other biomolecules) interaction in further detail. This is of emerging importance as more and more complex particle, vesicle and artificial liposome systems can be produced with their shell masking their contents. This is an

often desired property for drug-delivery amongst other applications, but needs to be understood also in the context of risk assessments. Notably the interaction of biomolecules with complex fluids within cells, tissues or organisms has been termed physio-chemistry and involves the complex interaction of the particle in its specific form as it changes during systemic exposure and migration with these fluids and the many bio-molecular components therein. Importantly physio-chemistry also involves physi**c**o-chemical steps like dissolution, chemical degradation and the passive (diffusive) transport, but goes far beyond in that there are specific bio-molecular adhesion and active transport mechanisms (also through membranes) triggered in response of such particulates with a cell, tissue or organism. One essential step which is directly related to the physical state of solid particles is the interaction of these particles as 3D objects with proteins and bio-molecules and is also referred to as 3D interaction. Another essentioal step is provided by the parallelity of different channels typically available for one stressor in an organism: Simultaneous to (a number of) uptake channels there are sublimation and degradation processes acting with a rate depending on the specific envoironment. The complex fate of engineered particulate systems within organisms imposes a new challenge for risk assessment.

Future potential

Increasingly efforts have been made to relate the well-known empirical connections between physico-chemical and structural properties to biodistribution and toxicity profiles via a more systematic scientific framework, where the structural and physico-chemical properties are related to microscopic biological interactions, and to the different modes of action. This could in future provide a more sound basis for predicting uptake, distribution and release of chemicals, and toxicity. This includes efforts to connect the detailed microscopic interactions between chemicals and their potential biological targets (for example protein, receptors) using high throughput screening (for example interaction, protein arrays and numerous others) rather than average properties, such as partition coefficients, that give only general indications of likely interactions. Such efforts are closely linked to the growing power of computational chemistry to predict and explain these interactions at molecular level. Still, these efforts have met with many challenges, and they are not sufficiently well developed to make reliable predictions.

The general approach may however be expected to become increasingly important. Thus, as the development of new materials (such as in nanotechnology) for applications ranging from energy recovery, storage, building, and many others accelerates there is often not yet sufficient scientific background on which to base predictions of biodistribution, clearance and toxicity. Also, simpler paradigms derived from molecules such as partition ratios are often insufficient to define the potentially very complex behaviour and fate of a chemical/material in an organism or the environment.

In the scientific community there is increasing belief that the key parameters of emerging materials are based on a wider spectrum of physico-chemical properties. The absence of relevant and simple physic-chemical parametrization together with the deficiencies of structure – property relations even for those chemicals which are currently in use, is significant for the future. Consequently, the need to more deeply understand the connection between structural and (potentially new) physicochemical properties and biological interactions could be the main route to predictive toxicology for new materials. Thereby new scientific knowledge is needed in conjunction with new tools to determine the predictive physico-chemical and structural properties. This issue will likely provide an essential scientific focus for the coming decades.

Conclusions

To date the predominant uses of physicochemical criteria have been to determine chemical stability in different environments and the nature and effect of impurities.

Structural parameters like the enantioform (chirality), but also the morphological form have been recognized to have decisive influence in some cases, the latter in particular with nanostructured materials. In addition to their use for the prediction of absorption data, lipophilicity and water solubility have been used as indicators towards one or the other distribution profile. Improved modelling of the interactions of individual chemicals with biologically important molecules (e.g. receptor proteins) in three dimensions in the medium term will contribute to predict more reliably the metabolic fate of chemicals and their modes of action.

5.5.Exposure assessment

Although a number of major advances in both chemical identification and quantitative analysis were achieved, exposure assessment remains the weakest part of the majority of human risk assessments. There are a number of reasons for this:

- In the development of the current risk assessment methodology the emphasis has been on improving hazard assessment.
- Human exposure assessments often rely on assumptions on consumer behaviour which is associated with a number of biases.
- Exposure assessment is a very complex discipline currently not an established academic discipline with a well-developed curriculum at major universities. Therefore, graduate and postgraduate training in exposure assessment is scarce and the number of experts is rather small.
- Investigations have tended to focus on single sources of exposure rather than on multiple sources which is the more common exposure situation
- Often exposure assessments have given insufficient attention to bio- and chemo-transformations of chemicals in the body or the environment.

With respect to the risk assessment of individual chemicals what is needed is a reliable estimate of the range of exposures that occur or are anticipated as a result of the manufacture, use (and potential misuse) and disposal (end of life fate) of each chemical or its transformation product (i.e. life cycle analysis).

When new chemicals are considered, modelling of exposure is the only feasible option. It is useful to make upfront a distinction between external and internal exposure levels, since this can make an important difference in a risk assessment strategy. External exposure can be defined as exposure of the external surfaces of the body. Internal exposure (dose) can be categorised into subsets:

- The amount of a chemical taken into the body by a single route or all routes;
- The metabolic fate of the chemical and its general distribution and excretion from the body;
- The amount of the chemical and/or its metabolites reaching a target organ(s);
- The uptake into the cells of the target organ(s) of the chemical and or its metabolites;
- The persistence of the chemical and or its metabolites in the body, determining the body burden.

Often only external exposure is considered.

Current approaches are mostly based on steady-state assumptions on chemical use or emissions such as described in TGD 2003.

5.5.1. Measurement of external exposure

External exposure for humans can be defined as the sum of:

- The amount of substance available to be ingested,
- The total amount in contact with the skin,
- The amount inhaled or the concentration of the substance in the atmosphere.

The measurement of external dose can either be done on stored samples, i.e. food samples or by direct measurement. The measurement of exposure based upon collected samples requires representative sampling and storage conditions that preserve the characteristics of the samples and applying appropriately sensitive, accurate and reproducible measurement techniques.

For some measurements however direct real time measurements can be made by monitoring major components of ambient air.

Samplers/ measuring devices for chemicals in air may be:

- At a fixed point, either for general monitoring (air, water) or to monitor a point source of exposure (e.g. a chimney stack), either continuously or through spot sampling. The most common application has been the sampling of food, water, soil, etc. This information can be useful for modelling external exposure.
- Mobile, e.g. personal monitors (attached to an individual). In a workplace, monitoring of exposure using both fixed point monitors and personal monitors is very common in order to ensure compliance of workplace air levels of a chemical with relevant regulatory standards.

Ambient air measurements for the criteria pollutants are also extensive throughout the EU in order to comply with the relevant directives. For chemicals in other media (e.g. food sources and drinking water), a range of programmes exist, again driven primarily by regulatory requirements.

Potential use in risk assessment

It is anticipated that in the future the regulatory requirements to measure chemicals in various media will increase significantly. Please refer to 4.1.2.3.2 for the challenges of monitoring activities: in particular the importance of evaluating average vs. peak concentrations, the spatial distribution of concentrations in the environmental media, the need of collecting data for chemical mixtures and nanomaterials.

To assess airborne exposure to chemicals, especially in the workplace, a particularly desirable development would be the availability of low cost personal samplers to enable individual exposures to be assessed. The increasing availability of better absorbents and advances in technologies such as 'the laboratory on a chip' make this a realistic prospect. The main issues are to ensure proper prioritisation based on which groups of chemicals to measure and in which media. The main barrier will be the availability of low cost high throughput measuring devices able to measure accurately a wide range of chemicals and their transformation products.

Regarding fixed point monitors, two trends are likely to continue:

- The continuous recording of real time data by remote sensing devices, and
- The development and increasing availability of high throughput, multi-component, analytical equipment based upon nanotechnology, or biosensors.

For measurements in other media the use of low cost high throughput analytical methods is likely to become very important. Development of highly sensitive validated and biologically relevant extraction procedures should be developed in case of consumer products in order to determine bioavailability.

Conclusions

Chemical measurements are likely to be an important contributor to tier 2 and tier 3 estimates of exposure. There are a number promising analytical techniques which should facilitate this.

5.5.2. Modelling of external exposure

Modelling of external exposure involves computer simulations to estimate exposure from the deliberate or accidental addition of chemicals to a particular media. The total exposure for a chemical is based upon the sum of the contributions from occupational exposures, food intake and use of consumer products as well as indirect exposure from the environment.

External exposure modelling is commonly used to estimate human exposure to a chemical or chemicals from new emission sources e.g. a proposed industrial plant by inhalation or through food. In addition, for consumer products, information on release data and use pattern are needed. These models are well established and typically have a high degree of built-in conservatism to allow for uncertainties associated with the modelling technique. Modelling is also used to assess transfer of chemicals through the food chain.

The main application to date has been to identify consumption of different foods and beverages. This has enabled estimates to be made of daily/longer term intake of the amounts of additives and contaminants by various consumer categories based on data on the likely levels of such substances in each food item.

According to TGD (2003a), external (indirect) exposure of humans via the environment may occur by consumption of food (fish, crops, meat and milk) and drinking water, inhalation of air and ingestion of soil. However, exposure via soil ingestion and dermal contact is not addressed in TGD. The indirect exposure is assessed by estimating the total daily intake of a substance based on the predicted environmental concentrations for (surface) water, groundwater, soil and air and using intake rates for each medium.

Additionally, TGD (2003a) calculates on two spatial scales: locally near a point source of the substance, and regionally using averaged concentrations over a larger area. In the local assessment, all food products are derived from the vicinity of one point source, in the regional assessment, all food products are taken from the regional model environment.

The procedures to calculate the external exposure in TGD (2003a) are coded in the EUSES model, similarly to what was described in the ecological exposure section. PECs for the environmental compartments in EUSES are obtained at steady state and in static environmental conditions (time invariant). Therefore, the model assumes constant emission of a chemical in a predetermined scenario which simplifies the fate calculation, but excludes temporal and spatial variability. PECs are then used to estimate concentration in food, drinking water and air. While many considerations already outlined are still valid in human external exposure assessment Several limitations of EUSES have been identified, e.g simplistic calculation of food concentrations as well as the poor predictive capability of the model in the regional scenario. Legind and Trapp (2009) integrate various approaches developing a "new model framework" based on a variety of crop models (instead of the generic plant model in TGD), suggesting that methods outlined in EUSES may lead to overprediction.

Since current approaches have focused mainly on adults and their exposure patterns, Trapp et al. (2008), using data from adult humans, developed a model predicting accumulation of POPs or other compounds in breast-feeding mothers and nursing infants after uptake of chemicals via diet or other relevant sources.

Another improvement over the simple approach in EUSES is that by Veltman et al. (2009), who developed a mechanistic bioaccumulation model for chemical exchange via

air, which is applicable to neutral organic chemicals and various mammals, using a limited set of data. The model was compared to measured data and it was shown that it produces a fit within a factor of 2–5 from empirical data.

The current EUSES also includes the prediction of consumer and workplace exposure which has its limitations.

Potential development

While EUSES calculates concentration in a fixed food basket, derived by contact with exposure media, the actual use of a variety of ingredients (often of animal origin) in animal feeds makes the evaluation of such concentrations difficult. For example, biomagnification in a terrestrial food chain (air-vegetable-dairy products and meat) could derive from chemical present in animal feed, not accounted for in the current approach.

Another issue of the current approach is the lack of an integrated mechanism to evaluate all the sources of a chemical and the consequent aggregated exposure of human beings: the mechanism implemented in EUSES simulates industrial chemicals and biocides, other approaches are developed with different categories of chemicals (such as plant protection products with the FOCUS approach), where human indirect exposure is calculated with different criteria (fixed concentration in groundwater, residue in crops) neglecting other environmental fate behaviours (movement in air, water and possible entry in the terrestrial of aquatic food web). Better models to consider contributions from consumer products based upon use pattern in different age groups and release of the compounds from the product (bioavailability)

Modelling in various forms, from semi-quantitative to quantitative estimates, is needed to provide the main input to exposure driven risk assessment (see below) and should considered changes in the exposure at different lifestages and accumulated exposure. In principle, external exposure modelling has great potential for further development. This would be greatly facilitated by the availability of exposure data banks. Assess to biological samples from the existing bio-banks will be critical in the validation of the modelling of exposure over time. Models will need to take more account of the chemical and biological transformations that a chemical may undergo in various media over time. It should be appreciated that the proposed introduction of Thresholds of Toxicological concern (TTC) for tier 1 assessments will generally rely on suitable exposure assessment modelling. The main advantage of modelling exposures is that the models can assess a range of scenarios in a short period of time. Models need to be properly validated and the assumptions used in the modelling need to be understood by stakeholders.

There are a number of challenges for improving models of external exposure: one descends from the need of accounting for variability in space and time of environmental concentrations (including food). This would allow to better estimate human variability in exposure, especially at different stages of the life cycle. Other issues (see also 4.1.2.4.2) are related to the many uncertainties in the understanding of the behaviour of polar chemicals, nanomaterials, mixtures, as well as bioaccumulation in food of different origin. There is also a need for a harmonized approach in modelling strategies for different categories of chemicals, such as industial substances and plant protection products.

In order to define the exposure scenarios a better understanding of lifetime activities would be a valuable aid. Developments should be directed to obtaining:

- Estimates of both typical and high exposures in different age groups and the factors that most influence this, i.e. use pattern
- Information on trends in exposure over time to particular chemicals of ‘concern’, due to societal or behavioural changes.

One method to assess exposure scenarios is the use of questionnaires and/or interviews to understand consumer habits and consumptions using e.g. time-activity diary. Such questionnaires will increasingly be available on line. The issue of incentives to fill them in accurately will need to be addressed. The benefit is that it is a readily applicable general

method to determine exposure of population groups. However, this methodology is subject to many biases e.g. recall bias, selection bias.

Modelling of exposure will only represent the external dose, but combined with PBPK this information can be transformed into the internal dose.

5.5.3. Consumer exposure (external)

Human exposure from chemicals in consumer products is a complex issue considering their huge number and uses. Tthe standard European model used for risk assessment, EUSES, for some consumer exposure calculations, requires the Consumer Exposure and Uptake (ConsExpo program) developed by the National Institute of Public Health and the Environment (RIVM) in the Netherlands. ConsExpo is a a set of models (ranging from screening models to higher tier exposure) developed for various exposure routes (inhalation, dermal or oral route. The ConsExpo program is integrated in the Euses program flow and includes appropriate data on release rate and modality, exposure scenario (variation in consumers and product use) and uptake information relevant for each route. Such default parameter values have been compiled in so-called fact sheets for a number of product categories (paint, cosmetics, children's toys and cleaning and disinfectant products, pest control products). It is currently used for the risk assessment within REACH and for Biocides. The most recent version is ConsExpo 4.1 (albeit a beta 5.1 version is available), which can be used to obtain insight in the factors affecting exposure levels to compounds in consumer product by using the tools of sensitivity analysis and probabilistic calculation. However, ConsExpo 4.1 is rather simplistic tool as it only describes a single chemical, single product, single scenario exposure assessment tool: the assessment can be performed for one chemical in one product selecting a specific pathway at a time. To assess aggregate exposure separate runs of the program are necessary.

Some concern were raised (Eickmann et al., 2007) for the spray model used by ConsExpo to calculate inhalation exposure. Additionally, when ConsExpo was compared with other existing consumer exposure modelling tools (Park et al., 2006) ConsExpo was shown to have the capability of running exposure scenarios comparable to those of the other models, while the calculation of aggregate exposure (exposure to a chemical present in multiple products) was not possible. Such criticisms, together with the fact that many default parameters (in the fact sheets) are based on Dutch conditions, constitute the main limitations of the model.

5.5.4. Modelling of internal exposure

As stated above, the knowledge of the physico-chemical features of a chemical can give an early indication of the fate of the chemical within the organism and contribute significantly to the understanding and prediction of internal dose in humans. Features such as hydrophobicity of a a chemical, determining its partition into living tissue, are crucial in absorption, the first step that controls the passage from an external dose of exposure to an internal dose. The first stage in the assessment of the internal dose is a reliable estimate of uptake from the intestine, lung or skin, depending on the route of exposure.

The processes related to the establishement of internal dose following ‘external’ exposure to a chemical is usually referred to as toxicokinetics (TK). Toxicokinetics integrates information about the absorption, distribution in the body, metabolism and excretion (ADME) of a toxic substance, and represent an essential piece of information for the appropriate design of toxicity tests and for data interpretation, being essential for e.g. interspecies and route-to-route extrapolation. Toxicokinetic data on metabolite formation may also contribute to explaining modes of action and their relation to dose level and route of exposure.

Assessment of TK data often involves both modeled and measured data, and is part of obligatory *in vivo* animal testing in some legislative frameworks (i.e. human and veterinary drugs, pesticides, biocides). The measurement of chemicals and their metabolites in body fluids (e.g. blood and urine) is commonly conducted in laboratory animals. In addition measurement of a chemical and/or its metabolites may be conducted in various body organs. Where a radiolabelled version of the chemical of interest is available it may be used to determine the total amount of chemical and its metabolites in laboratory rats or mice. Whole body autoradiography of rats and mice may be used to visualise the body distribution of the radiolabel.

'Standardized' toxicokinetic testing in experimental animals is described in the updated OECD test guideline 417, which clearly indicated *in vitro* and *in silico* methods to measure various ADME parameters as an adequate tool to substantially reduce animal testing. So far, toxicokinetic data related to absorption and metabolism can be generated with non-animal testing with *in vitro* or *in silico* approaches, whereas measurements of chemical distribution and excretion are only rarely conducted *in vitro*, due to lack of reliable methods.

As the reduction in animal use is a major driver for change, an increased use of *in vitro* systems is foreseen to identify uptake, distribution (including subcellular distribution), metabolism and clearance in cells and other preparations of externally added chemicals. This is likely to be especially relevant for materials such as nanomaterials.

Unless such measurements are conducted, confidence in *in vitro* to *in vivo* extrapolation is at present unlikely to be achieved. The most promising way to overcome problems encountered in the *in vitro*-*in vivo* extrapolation is the use of modelling. The advances in the data sets used for model building, the molecular descriptors, the prediction models, and the statistical modeling techniques, have been repeatedly summarized (Clewell and Clewell, 2008; Hou et al. 2009,Bois et al, 2010). Experimental data can be used in a population based *in vitro* to *in vivo* extrapolation model to estimate the daily human oral dose – necessary to produce steady-state *in vivo* blood concentration (Wetmore et al., 2012), (Rotroff et al., 2010). But the most appropriate tool is at present represented by Physiological based pharmacokinetic (PBPK) models.

There are data rich models predicting tissue concentrations of a xenobiotic in humans or experimental animals (Hou et al. 2009)(. Up to now, PBPK-models have been used to improve risk assessment by supporting the derivation of extrapolation factors when inter-species, inter-dose or inter-route extrapolations are performed. PBPK models have the potential for extrapolation from observed kinetic data to predicted scenarios and therefore make possible the extrapolation of results from laboratory animals to humans, from observations at high doses to low doses and from single to continuous exposures. Up to now, they have allowed prediction of potential species or inter-individual differences in humans by integrating the toxicokinetic and toxicodynamic factors causing these potential differences (Bois et al, 2010). Therefore PBPK-models are most valuable for any proof definition and justification of the extrapolation factors used in risk assessment when defining "safe" or tolerable exposures. PBPK-models may also predict interactions in mixtures, providing that the modes of action are known. The expansion of application of PBPK-models will result in more precise quantification of tolerable exposures.

Although used in an increasing number of different areas, they are often perceived as complex, resource intensive, and time consuming due to the requirement of model validation and verification. The development of web applications for the rapid construction and documentation of deterministic PBPK model could help to overcome these concerns (Loizou and Hogg, 2011).

In order to give a reliable output, high-quality data are needed as input for these models. Physiological parameters as well as physico-chemical properties are usually available, whereas a number of chemical specific toxicokinetic parameters such as partition coefficients and metabolic rates by different enzymatic reactions are not. Generation of

these data *in vivo* is time-consuming, although the possibility to use *in vitro* data as input for PBPK modelling could partially solve this problem. The limitations of *in vitro* toxicokinetic assays have an impact on the predictive accuracy of PBPK models. Intrinsic to PBPK modelling itself is the difficulty to accurately model dermal exposure (e.g. surface area exposed, dose applied wearing and washout) and absorption (e.g. saturation of the skin layers). If reliable data become available, their input in human PBPK models can provide tissue specific concentration estimates for comparisons with human biomonitoring programs.

The development of in vitro ADME models has taken place essentially in the pharmaceutical R&D area (Blaauboer, 2010), which has used values for isolated processes to feed (parameterize) PBPK models (Adler et al., 2011). Thus, assessment of oral bioavailability and bioavailability-related properties, especially intestinal absorption are areas in need of progress, not only to aid pharmaceutical drug development but also for other purposes, such as to stop any testing for systemic effects in cosmetics, in the absence of any absorption (Adler et al, 2011; Coecke et al, 2012). As a consquence Development of reliable in vitro models for absorption is important as a component of a tier 1 exposure assessment, and as an input to PBPK modeling.

A number of *in vitro* and *in silico* methods have already been developed for screening purposes to measure oral absorption and bioavailability (Adler et al., 2011), however in order to use the *in vitro* absorption results as input parameter in PBPK modeling, more efforts and developments are needed. As an example, the CaCo2 method which is most widely used for identifying the passage across the intestinal barrier, shows limitations in prediction of oral absorption whenever highly lipophilic compounds or substance in the low-to-moderate absorption area (<30%) are tested as well as when transporter-mediated routes and/or presystemic metabolism are involved (Turco et al, 2011).

In vitro alternative assays for the dermal absorption barrier have been developed and translated in the OECD test guideline 428. Although already used in some regulatory framework (i.e. plant protection products) to predict the internal dose after dermal exposure, its use is limited by the scant biotransformation capacity of the biological system used.

The methods for absorption across pulmonary epithelial barriers, e.g. the inhalation route, are at present at an early stage and should be further developed. This would provide valuable input into PBPK modelling and inform the need for any in vivo assessment. In regard to nanotechnology products the in vitro models or the use of a complementary *in silico* model need to consider regional dosimetry due to the deposition of particles in the respiratory tract.

Regarding metabolism, the *in vitro/in silico* available methods are in a good stage of development and in many cases they can already provide information relevant for risk assessment and of sufficient quality as input for PBPK modeling. They have enabled the primary enzymes responsible for metabolites to be characterized and genetic variations in some of the enzymes to be identified. However, one challenge is when the metabolism depends on several organs. Apart from protein binding in vitro methods for distribution are rather poorly developed. Also the excretion phase is not yet sufficiently covered by *in vitro* methods: indeed with the exception of glomerular filtration rate, this has been identified as one of the major data gap (Adler et al., 2011)

The importance of kinetic data has an impact also in the possibility for a future replacement of in vivo testing. Indeed, one of the key factors limiting this change is to replicate the ADME factors *in vitro*. While substantial progress in this regard has been made in terms of absorption and metabolism this is not the case for distribution or excretion. In the design of *in vitro* tests the presentation of the chemical to the test system to reflect the *in vivo* situation is very important. Again modelling can provide the answer to address the issue, but in order to relate toxicodynamic information from *in*

vitro testing to (human) *in vivo* situation, it is necessary to investigate the kinetic of chemicals in the *in vitro* toxicity testing system used. In other words it is necessary to produce data on *in vitro* disposition and concentration-time curves for both the parent compounds and/or its metabolites. *In vitro* biokinetics data provide the actual level of cell exposure producing the observed effects and improved the interpreation of dynamic data. On the issue an EU funded Project (PredictIV) is presently going on producing very promising data (*www.**predict-iv**.toxi.uni-wuerzburg.de/*). Results clearly indicate that kinetic data are necessary to convert the *in vitro* results into dose response or potency information relating to the entire target organism (in vitro-in vivo extrapolation), through the use of appropriate modelling systems. Therefore, for the proper design and performance of in vitro studies, it is important to include kinetic and analytical aspects in the *in vitro* test methods. The main problem in the development of *in vitro* kinetics is the difficulty of measuring free and internal cell concentrations in *in vitro* systems determined by both abiotic (i.e. chemical stability of the compound over time, adsorption to the plastic devices, evaporation, binding with the medium components) (Gülden et al., 2001; Heringa, et al., 2004; Kramer et al., 2009, Kramer, 2010), and physiological cellular processes (transport across the membranes, biotransformation, bioaccumulation). However, the as yet limited experience in this specific field requires further development and refinement.

The progressive increase in the understanding of mode(s) of action is foreseen as strengthening the application of PBPK modelling for risk assessment purposes. Information on genetic variation, genetic polymorphisms and age sensitive could be included in the modelling, under the conditions that the effects of polymorphisms and of age specific expression of the genes are known.

PBPK modelling is likely to be a very important component of a tier 3 exposure assessment. PBPK modelling can be used in the tier 3 to decide whether further data on toxicity may be needed regarding characteristics and routes of exposure.

Considerable uncertainties still exist on the applicability of such models for certain categories of chemicals such as polar or ionised and/or nanoparticles and further development is needed in this area.

To validate PBPK-modelling, toxicokinetic studies may also be performed in humans: information as to the likely metabolism of a new drug can be determined at an early stage through the use of microdosing in human volunteers. In such studies, very low doses of a chemical (about 100-fold lower than the anticipated minimum therapeutically-active dose for a new drug) are administered to humans to mimic the actual exposure conditions. Microdosing, in association with sensitive accelerator mass spectrometry (AMS) technology, has been shown to be capable of providing valuable insight into the way a drug is likely to be handled by the body when dosed within the therapeutic range. To this purpose the use of stable isotopes is often required for the specificity of modern analytical procedures. Stable isotopes allow specific chemicals to be specifically labelled. They are considered to be preferable to radioactive isotopes because of real or perceived health risks. They require the use of mass spectrometry as a sensitive and specific analytical technique. Indeed, the presence of a stable isotope and the resulting specific signature in mass spectra permits the determination of the chemical of interest and its relevant metabolites even in the presence of background exposures. As the use of doses inducing toxic responses is unethical, only human studies using stable isotopes and very sensitive analytics can be used as they only need to apply very low doses. Regarding occupationally and environmentally occurring chemicals, the use of studies in humans has generated a number of relevant data for confirmation of PBPK-models and general use in risk assessment (Poiger and Schlatter, 1986; Schauer, 2006).

Such studies may give precise information on the toxicokinetic of an agent in humans, including variability within the population and route-dependent differences although no information on toxicity endpoints or hazard assessment is provided. Due to the ever increasing sensitivity and specificity of modern analytical chemistry, very low

concentrations of chemicals can be determined even in complex matrices. Therefore, studies on the toxicokinetic of very low doses of chemicals in humans will be increasingly feasible, although studies with stable isotopes require sophisticated techniques and specific quality control that make them time-consuming and cost-intensive: therefore they can only be applied to selected chemicals with specific applications.

Stable isotopes should be one of the tools available for tier 3 exposure assessment. For ethical and other reasons a much more extensive use of this technique is not envisaged.

Conclusion

TK represents an essential piece of information for the appropriate design of any toxicity tests and for data interpretation. The use of physico-chemical data allied with some simple *in vitro* tests for the estimation of uptake from various routes of exposure, metabolic fate and persistence of chemicals in man is particularly important for tier 1 exposure assessments. Such data will also be needed for tier 2 assessments. This would enable the application of SAR. The production of *in vitro* kinetic data in tier 2 *in vitro* toxicity testing is essential for the *in vitro-in vivo* extrapolation for which PBPK modelling is considered as the most appropriate tool. PBPK modelling is likely to be also a very important component of tier 3: for a refined exposure assessment, for the estimate of effects in vulnerable populations and for the evaluation of mixture effetcs. Considerable uncertainties still exist on the applicability of such models for certain categories of chemicals such as polar or ionised and/or nanoparticles.

5.5.5. Measurement of internal exposure (biomarkers)

Biomarkers have been developed and used in environmental health to enhance exposure assessment, gain insight into disease mechanism, and better understand susceptibility, i.e., gene-environment interaction. Furthermore, they have been used to address issues such as cumulative health risk from exposures to multiple environmental stressors, including aggregate exposures. Historically the focus has been on genotoxic compounds.

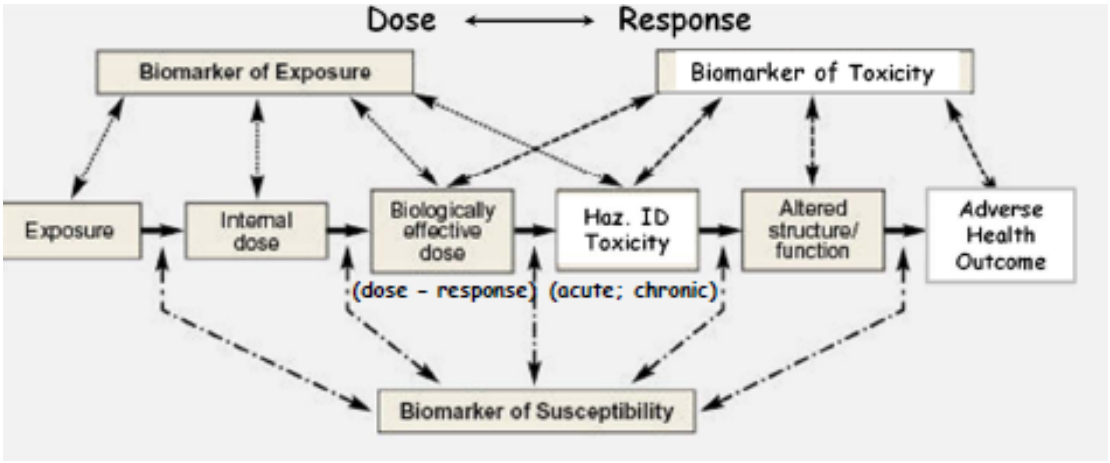
Definition of biomarker varies across scientific fields (toxicology, occupational hygiene, medicine and epidemiology). According to the World Health Organization (IPCS, 2001), a biomarker is any substance, or its metabolites, structure, or process that can be measured within an organism and influences or predicts the incidence of harmful effects or disease.

The WHO identifies three classes of biomarkers:

- Biomarker of exposure: the chemical substance, its metabolite or reaction product with cellular macromolecules.
- Biomarker of effect: a measurable biochemical, physiological, behavioural or other alteration within an organism that - depending upon the magnitude - can be recognized as associated with an established or possible health impairment or disease;
- Biomarker of susceptibility: an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance.

The concept of biomarkers is illustrated in Figure 4.

Figure 4: The various types of biomarkers



The biomarkers described in Figure 4 represent different stages in the pathogenesis from exposure to adverse health effect. Whereas the biomarkers of exposure (internal dose and biologically active dose) may represent exposure for a specific chemical or its metaboliteThe toxicokinetics of the compound should be taken into consideration when designing a study using biomarkers of exposure, as they reflect not only the exposure but also the individuals response, e.g. biomarkers of susceptibility. Generally, most biomarkers of effects do not reflect the identity of the chemical causing the effects as this type of biomarkers will be influences by other effects such as nutritional factors and other environmental as well as endogeneous factors. Thus the biomarker of effect may be more related to the early phenotype of the disease than the identity of the chemical causing the disease. Human biomonitoring of chemical exposures is currently used in the characterization of health risk under REACH (Boogard et al., 2012) A bioindicator is a specific biological marker of effects that represents a key event for the development of a particular disease, and is therefore more relevant for MOA based risk assessment.

Translation of concentrations of a chemical in blood or urine of individuals to an external dose that is the basis for regulatory practise can only be performed if the toxicokinetics of the chemical are well described. Based on the toxicokinetics of the chemical, the monitoring strategy regarding sample collection intervals, number of individuals sampled, sample type (urine, blood or other), and analyte to be determined (parent compound or metabolite) needs to be developed. For rapidly metabolized chemicals, a large number of individuals need to be sampled to "average out" the expected large variations in analyte concentrations over time or specific sampling strategies need to be developed (e.g. at a specific time after a meal).

A significant challenge is associated with monitoring as well as assessing individual and population level exposures related to age and lifestage related changes in behaviour and physiology. These changes will determine the critical windows of susceptibility as well as the windows of highest exposure. The WHO has developed guidance on how to identify systematically critical life stages for use in exposure and risk assessment (IPCS, 2011).

Future potential

Biomarkers have the potential to provide information on aggregate exposures, multiple sources and multiple pathways.

The development of new chemical analytical technologies and the rapid advances in omics (see below) are likely to result in the identification of many new biomarkers. A crucial point in the application of –omics biomarkers in exposure assessment is the need of establishing a qualitative and quantitative relationship between the exposure and the –

omic profile. Other sensitive technologies include inductively coupled plasma mass spectroscopy (ICP-MS), fluorescence in situ hybridization (FISH) and various omics technologies. Other technologies include imaging, e.g. PET, CT and MRI.

Advances in the understanding of modes of action of chemicals should enable the identification of biomarkers for the various stages in the development of chronic adverse effects. As a result it will in principle be possible to identify biomarkers for the early stages of the development of toxic effects and thus useful for carefully controlled volunteer studies in man in the future. Biomarkers could also be used to identify individuals with high exposure, e.g. accidental exposure to chemicals. Biomarkers of exposure should also enable the assessment of the dose-response relationship in a more relevant part of the curve because of their greater sensitivity and, potentially, their precision. The use of the next generation sequencing could be used to identify somatic profiles or signature mutations linked to environmental exposures, i.e., exposure classification. The use of epigenomic information may be useful to identify changes during development and their subsequent role in the development of chronic diseases.

Informative biomarkers are those that are persistent, e.g., long half life, are easily collected using non-invasive procedures, and can be used to establish a link between exposure and disease. Biomarkers should also have sufficient sensitivity to give information on regional differences and differences in time scales. In addition they should preferentially reflect the mechanism of action of the chemicals. A reverse dosimetry approach can be used to estimate the daily exposure.

Development of sensitive and robust biomarkers that link environmental exposures to the pathogenesis of human disease is a leading priority in the field of environmental epidemiology. It is important to develop biologically based exposure metrics in order to interpret the emerging toxicity data and advance human health risk assessment. This would require application of environmental informatics capabilities and advanced computational tools to model and link exposures to health outcome. A systems exposure framework links key events in the toxicity pathways to characterize stressors and the processes that will lead from the exposure to dose at the critical target pathways. The exposure network consists of 3 tiers: 1) exposure pathways to circulating blood 2) mechanism of metabolism that produce reactive shortlived metabolites that could be measured as biological parameters (biomarkers), and these biomarkers can then be linked to markers resulting in system perturbations (Pleil and Sheldon, 2011).

When using omics to measure biomarkers, a problem is the specificity and temporality of the link between exposure and biomarker response. Similarly, the metabol(om)ic fingerprint is dependent on the dose. Furthermore a concern could be a choice of technology platform as well as natural variation due to e.g. diet.

Biomarkers have the potential to generate precise and reliable exposure data for chemicals or their metabolites when integrated into biomonitoring studies (biosurvey). These types of studies will be useful to track population trends, to identify susceptible populations, to provide indications of emerging environmental health issues, and to monitor effects of mitigations. They may therefore lead to a significant improvement of exposure assessment and permit a precise assessment of exposures at targets of toxicity when toxicokinetics are known. The measurement of environmental chemicals in an individual's blood or urine does not indicate a risk for disease. Without information on some of the key molecular events associated with the chemical exposure, caution in the interpretation of these data for risk assessment is recommended.

The integrated application of human biomarker data on exposure and susceptibility into epidemiological studies can provide more solid data in relation to the human health risks resulting from exposure to environmental toxicants, i.e. molecular epidemiology. Most of these studies have focused on markers of susceptibility, whereas markers of exposure and biomarkers of effect have only been used to a limited extent, e.g. carcinogen-DNA adducts and chromosomal mutations. These biomarkers have been used in several

studies based upon biobank materiel in a nested case control design, and the utility of this approach has been reviewed in monographs from the ECNIS study.

The use of biomarkers in epidemiological studies to classify exposure will improve the relevance of these human studies in quantitative risk assessment. Biomonitoring is considered the "gold standard" of exposure assessment for environmental chemical exposure; however most of the data is based upon a snapshot of the internal dose. These biosurvey data can also be misused by associating effects in cross-sectional studies when based on single-point measurements.

Future prospects for application of biomarkers in health risk assessment are promising, if the biological relevance of the biomarkers could be established, i.e. by knowing the mode of action. The expected advances for the coming years are: increased reliability in the exposure assessment and detection of early harm in populations exposed to low doses and to the complex mixtures of chemicals, as well as, increased sensitivity. The information from population surveys using biomarkers could be useful for a public health point of view and for preliminary hypothesis generating studies.

The advantage of biomarkers is that they represent aggregated exposure considering multiple sources and pathways (oral, inhalation and dermal) of the same chemical.

Numerous factors in addition to the exposure influence the biomarker levels and results in a large variation. Barriers between areas of knowledge need to be eliminated in order to ensure proper use of biomarkers in the context of health risk assessment The major problem using biomarkers to assess exposure is due to the variation, including both inter-individual (inter-subject), intra-subject variation (i.e. temporal variation), biological sampling and laboratory variation. Intra-subject variation is dealing with the meaning of altered levels of predictive biomarkers at an individual level. Based on current knowledge, the inter-individual variability in toxicokinetics and toxicity is extensive. The role of genetic susceptibility in health risk assessment was addressed in the paper by Knudsen *et al*. (2001), that questions whether the safety factor of 10 will be acceptable when one begins to examine multi-gene–environmental interactions. However more recent studies indicate that the factor 10 will be sufficient. The role of genetic variation depends on the exposure level and is most likely to play a role only at high concentrations. Another crucial aspect is the validation issue: only few biomarkers have undergone the vigorous validation process up to now. A proper validation of biomarkers can enhance health risk assessment and contribute to effective prevention policies in environmental and occupational settings. In principle, the process of validating biomarkers involves dealing with a range of characteristics that include the intrinsic qualities of the biomarker, its determinants, and the analytic procedure. According to the WHO (IPCS, 2001), validity refers to the accuracy of the biomarker; it is a complex characteristic that describes the extent to which the biomarker reflects a specific event in a biological system. A biomarker can only be used systematically after experimental and epidemiological validation.

The development of highly sensitive and specific exposure biomarkers requires new analytical approaches using high throughput techniques. A problem is the availability of biological material, e.g. if invasive methods are required the sampling may not be representative of the whole populations. Furthermore, analysis of chemicals and their metabolites in accessible biological index media may not provide the necessary information about the cellular target dose. Therefore, surrogate effect markers are needed. The use of markers that can be detected in blood, urine, saliva, sweat or breath is therefore a priority for routine screening of population groups.

Systemically collected relevant biological material in biobanks will be a potent tool to assess exposure and early biological effect using biomarkers will be important in future risk assessment.

Conclusions

Biomarkers are anticipated to play an increasingly important role in:

- The validation of test systems including the appropriateness of various *in vitro* models for specific toxicity testing purposes;
- The early detection of effects that in the longer term may result in marked adverse effects.
- Identification of vulnerable populations
- Analysing risk of aggregate exposures – multiple sources and pathways

Disadvantages are the high cost, and time consuming analytical procedures. Depending on the type of biomarker, there may be a concern about the lag-time between exposure and biomarker expression, e.g. measurements of chemicals with a short biological halflife or unstable metabolites. Moreover, not considering toxicokinetics when performing biomonitoring will result in useless data, e.g. when concentrations of a rapidly metabolized and excreted chemical collected only at one sampling point are considered indicative of overall individual exposure and are claimed to relate with potential effects in a population.

Currently the biomarker work focuses on chemical of high scientific or public interest, with little focus on natural components in food with significant potential for toxicity.

As molecular level toxicity pathways may be used to drive future risk assessments, there is a critical need for exposure assessments at the molecular level as well. In addition, different classes of biomarkers are required in order to integrate the information into the toxomes concept of risk assessment. Monitoring using high throughput methodologies, e.g. lab on the chip, will increase the possibility to monitor complex mixtures.

5.6.Hazard assessment

5.6.1. Studies in man

Introduction and current use

The gold standard for risk assessment is reliable information on the effects of the chemical and non-chemical stressors in man. The information can be obtained from well-designed epidemiological studies or properly conducted controlled human exposure studies. For ethical and practical reasons most information on the effects of chemicals in man comes from a retrospective analysis. The main exceptions are medicines and personal care products where the benefits are considered by those exposed to outweigh the risks and situations where prospective studies are possible because exposure of a group of people is currently occurring and is likely to continue.

Several types of study can provide information on the effects of chemicals:

- For acute exposures, information from poisons centres about events such as chemical spillage/release incidents, accidental/deliberate poisoning. Volunteer studies can be performed for drugs, personal care products and some other chemicals using low doses
- For chronic exposures - epidemiological studies (workplace, local population and wider population groups).

Our knowledge of the health implications of exposure to a number of chemicals and chemical combinations comes primarily from epidemiology studies (e.g. tobacco smoke, asbestos, lead). Human epidemiology data will continue to be the primary source of adverse effects data on complex mixtures of environmental chemicals, e.g. ambient air. However, most of the information is from high exposure (i.e. occupational) situations not relevant for the general populations. In the REACH regulation, quality criteria for epidemiological studies has been established (Annex 9), generally based upon the Bradford-Hill criteria. Similarly, guidelines for reporting different styles of epidemiological

studies have been established "The Strengthening the Reporting of observational Studies in Epidemiology (STROBE)" (von Elm E et al., 2007).

In recent years, advances in sensitive laboratory techniques have led to a rapid increase in the use of biomarkers of susceptibility in epidemiological studies, a field described as molecular epidemiology. These studies integrate molecular markers of exposure and susceptibility into an epidemiological design. The focus on most of these studies has been on genotoxic compounds and genetic polymorphisms in genes playing a role in gene-environment interaction, e.g. metabolism, transport and DNA repair. Most of the studies have taken advantage of the established cohorts in Europe.

A very substantial amount of information on the adverse effects in humans is available in the area of medicines. There is also much information in company files on the dermal effects of various chemicals during volunteer studies. Such studies in a toxicology context in humans only cover observational work on reversible and subtle effects which may be observed by techniques such as magnetic resonance imaging (MRI) or to study toxicokinetics of agents after applying low doses, well below effect doses.

For such purposes clear criteria for termination of a study have to be included in the study protocols. The threshold for adverse effects of acute respiratory irritants such as nitrogen dioxide and sulphur dioxide and other substances such as alcohol have been identified as a result of limited volunteer studies.

Future potential

The main advantage of human data is that the information obtained is directly relevant and removes the uncertainty in extrapolation from animal and *in vitro* tests. The disadvantage is that the exposure levels in humans cannot be controlled except in the very short term. Those confounding factors become increasingly important as the duration of exposure increases.

It is vital that a suitably validated database is developed for the acute and chronic effects of chemicals in man. This would be valuable for:

- Validation of *in vitro* and *in vivo* tests;
- Confirmation of mode of action studies;
- Validation of read across systems;
- Identification of human variability in response to individual chemicals.

In regard to future studies, the development of suitable biomarkers of the early (and reversible) stages of the development of chronic adverse effects along with reliable estimates of likely human exposures could justify carefully controlled volunteer studies for a substantially wider range of chemicals (see sections on biomarkers and microdosing).

New and sensitive markers of exposure and effects can be integrated into an epidemiological design, e.g. omics technologies. In addition, information on the epigenome and its variation over time and as a function of lifestyle and environmental exposures could be integrated.

New technologies will also provide new information on the variation in DNA sequence and the genetic basis for inter-individual variation in response to chemical will be explored giving a more scientific basis for determination on the safety factors currently used.

Information on human effects could be improved by considering the large databases of unwanted effects from pharmaceuticals testing.

The primary barriers other than ethical considerations are:

- Lack of full publications on studies in man. This is an important issue to address in the near future.
- Costs and other logistical issues (e.g. finding suitable volunteers).

Conclusions

Information on the effects of chemicals in man is highly desirable. A validated data bank of such information is needed. Ethical constraints are likely to limit prospective studies although new early markers of chronic disease may permit some carefully controlled studies in human volunteers to take place. Such markers could also be a component of routine health screening in the future.

5.6.2. Studies in animals

Toxicity testing for regulatory purposes is usually performed following OECD testing guidelines. For certain purposes, specific rodent models have been developed to reduce the time required for the study (e.g. genetically modified animals for carcinogenicity testing). The models now available have been applied for the last 30 years and are continuously refined to include new endpoints. However, these endpoints need to be validated and also correlated to an adverse effect. Risk assessment procedures dictate that the route of administration be relevant to the routes of human exposures. However, mixed exposures such as inhalation and oral exposure to the same chemical are difficult to simulate; moreover, dermal administration is difficult for long-term experiments in the hazard assessment since it often requires very high local doses.

Some recent test systems use genetically altered or "transgenic" mouse models which carry activated oncogenes or inactivated tumour suppressor genes known to be involved in cancer development. The genetic modification may result in a more rapid response to carcinogens as compared to conventional rodents. At present, two models, the p53 deficient and Tg.AC (v-Ha-ras transgene mouse model) are potentially useful to identify carcinogens and mechanisms of action. These models have been applied by the US National Toxicology Programme to study the carcinogenicity of a limited number of agents which gave questionable results in standard carcinogenicity tests.

Future potential

Advances in understanding of the roles of individual and groups of genes in humans and experimental animals and improvements in the techniques for gene manipulation will undoubtedly occur. As a consequence, the potential to develop new animal models that reflect human disease and other causes of individual variations in response to stressors is very large. Due to the slow progress in developing non-animal approaches for hazard assessment and the considerable uncertainties or even the absence of agreed procedures for risk assessment based on in vitro data, it is possible that OECD-guideline adherent animal studies will remain a significant pillar. However, a critical analysis of the benefits of performing all required studies should be performed and studies not providing usefull information should be removed form the regulatory requirements.

Conclusions

The use of specially bred animals for testing purposes (particularly genetically modified animals) has a high potential both for elucidating modes of action and for reflecting particularly vulnerable population groups. However this potential is likely to be very limited within the European Union by political/ethical concerns. For risk assessment purposes animal experiments are likely to be required in tier 3.

5.6.3. *In vitro* studies

There are large and increasing political pressures in the EU to replace *in vivo* tests with alternatives. In seeking suitable alternative tests it is essential to appreciate that individual biological systems are complex with many positive and negative feedback mechanisms to preserve homeostasis. Replicating these many complex processes in an *in vitro* or *in silico* model is very challenging and depends on the availability of a profound knowledge of the key components of these systems. As a consequence, progress in the development of suitable test systems to mirror various *in vivo* endpoints has been inevitably slow. When considering priorities, it is essential to distinguish between alternative tests that simply replace *in vivo* tests for a specific endpoint and alternative tests that need to be developed to improve on deficiencies in our current testing procedures. To date almost all the emphasis has been placed on the former.

The essential goal of *in vitro* studies is to mimic the effects of a chemical *in vivo*. This should include the dose-response relationship, which implies the need to investigate the kinetic of chemicals in the *in vitro* toxicity testing system used, i.e. to produce data on *in vitro* disposition and concentration-time curves. *In vitro* tests using microorganisms and animal/human cell lines has achieved widespread use for the identification of genotoxic properties of chemicals, for studying specific mechanisms, for screening purposes and more recently some recent significant advances have been made in the use of *in vitro* tests to predict acute local toxic effects. However, the progress on tests to identify systemic effects (acute as well as long term) is so far very limited. Simply determining cytotoxicity in a cell culture gives only very limited information on the types and severity of effects to be expected from a stressor in an intact organ since most of the test systems use cultured cells derived from a specific cell type in the organ of interest. Interactions on a tissue level, which may be major contributors to the development of a toxic response, can rarely be assessed and the development of 3D cell models could represent a substantial improvement.

Priorities for the development of such models need to be developed. If the ultimate objective is a complete transition from *in vivo* to *in vitro* to *in silico* in order to reduce the use of animals with the same or higher level of confidence in the safety evaluation, it is important to identify pathways and constraints in achieving it. At present the switch from *in vivo* to *in vitro* is already very difficult since it is not yet possible to link exposure in cell cultures to internal dosing (or toxicokinetics): in this respect *in vitro* biokinetics data providing the actual level of cell exposure producing an *in vitro* observed effects can improve the extrapolation, specifically after repeated dose applications as needed for risk assessment, with the aid of PBPK and PBPD modelling. However, the limited experience in this specific field requires further development and refinement.

Scientific experts in five toxicological areas, i.e. toxicokinetics, repeated dose toxicity, carcinogenicity, skin sensitisation, and reproductive toxicity (the area for which the Cosmetic Directive foresees 2013 as the deadline for the stop in animal testing) were asked to analyse the status and prospects of alternative methods and to provide a scientifically sound estimate of the time necessary to achieve full replacement of animal testing (Adler et al, 2011). A period of 7–9 years was estimated for the replacement of the current *in vivo* skin sensitisation testing. However, this time frame will only generate information content on hazard, since the relative potency of a sensitiser cannot be determined. No estimation for the time needed for a full replacement of animal tests in the area of systemic toxicological endpoints (repeated dose toxicity, carcinogenicity and reproductive toxicity) was possible. For toxicokinetics, many experimental models are available and already used, but each one can give information only on a specific ADME step: the timeline needed to integrate the results could not be given (Adler et al, 2010) and represent the challenge for the near future.

Although some progress has been done in the past years, the challenges are to make available cell cultures/systems, possibly as 3D models, with adequate stability over time (i.e. having characteristics of their *in vivo* counterparts for some weeks to several months, including the relevant panel of drug metabolizing enzymes), These cell models,

each of them giving specific information (e.g. organ/tissue-specific toxicity) have to be combined in an integrated battery able to be predictive for the human health protection, likely using PBPD modelling. To reach this goal it will be also necessary to understand the actual meaning of changes in biochemical pathways observed *in vitro* (and/or alterations detectable with 'omics' techniques) and their translation into adverse effects *in vivo* with potential role in the development of organ pathologies.

Similarly the Adler et al. report outlines a framework for risk assessment without animal testing in which two issues are crucial: 1) the central importance of toxicokinetics in the design and conduct of toxicological (*in vitro*) tests and the interpretation of toxicity data in the extrapolation from external to internal exposure and from *in vitro* data to the human *in vivo* situation; 2) the identification of the extent of exposure (including exposure scenarios for multiple routes and multiple sources).

Future potential

There is enormous potential for the use of *in vitro* tests for risk assessment purposes. However it is important to identify whether the strategy should be to find an alternative to each important endpoint in vivo or whether a more integrated approach should be the focus for development. One very important contribution of *in vitro* models to the future paradigm should be their use to identify and characterise modes of action (see below).

There are a number of important prerequisites to realising this potential. For example:

- Methods for the presentation of a range of stressors to the test system in a form that reflects the *in vivo* exposure situations
- Greatly improved understanding of the pathways (modes of action) that lead to significant toxic endpoints. This will enable early markers of toxicity to be determined in the *in vitro* preparations.
- *In vitro* models that can be standardised and which reflect human responses to a range of stressors.

Particular attention needs to be given to the identification of the cellular or tissue requirements for particular types of hazards. For a number of hazard identification screening purposes it may not be important that the cell, tissue or organ in the preparation has lost a number of its *in vivo* characteristics. However, this is unlikely to be the case generally. The use of human stem cells has been proposed as a potential solution to the quest to replicate *in vivo* cell properties *in vitro*. Some promising studies using human stem cells for specific purposes have been performed (Sison-Young et al, 2012; Kia et al, 2012). However, their real potential for chronic hazard characterisation has yet to be established. Advances in systems and synthetic biology may be expected to play an important role in the development of new *in vitro* models.

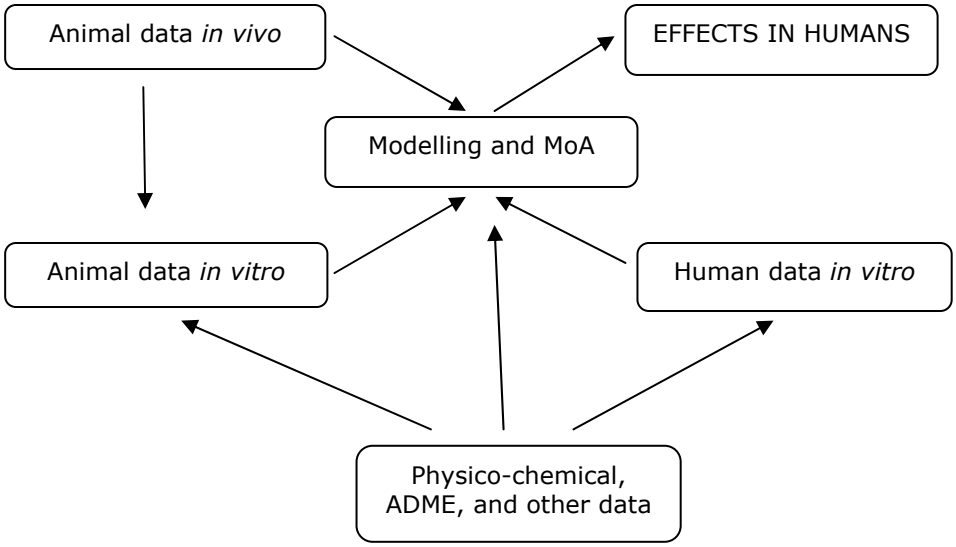
From a risk assessment perspective the priorities for the development of *in vitro* test systems are:

- Methods that allow the actual *in vitro* cell exposure levels to be related to the in vivo exposure levels. This requires both understanding of uptake of each chemical by the in vitro preparation but also that the preparation enables the in vivo kinetics to be reflected in vitro. In addition the relative sensitivities of the in vitro preparation and the live animals/humans must be considered;
- *In vitro* models need to be developed that maintain the in vivo characteristics of the organ from which they were derived for a minimum of several weeks. The *in vitro* preparations needed will include both isolated cells and organs/tissues or mixed cell preparations representing the cellular makeup of the tissue /organ;
- Ensuring that due cognisance is taken of the current advances in the understanding of biological processes including mode of action findings;
- Establish a validation process that is reliable, valid and prompt. This requires a suitable data base for such comparisons to be made;

- A clear strategy for the use of different *in vitro* preparation that reflects the needs of the risk assessment paradigm. A tiered approach is proposed for hazard assessment (see below). It will be important to consider where each development and proposed development fits in this tiered paradigm.

To bridge the gap between animal data and human data where there is no data in man and it is viewed as unethical to obtain relevant human data is to compare the findings from animal and human derived cells (Figure 5). Where substantial differences are observed between human and animal cells then some in vivo studies in the animal should be conducted to characterise further the effects of concern where the substantial differences occur.

Figure 5: An *in vitro* led approach to human risk assessment



The figure identifies that the starting point for the assessment of the properties of a specific chemical is to examine the physicochemical properties. This information would be used to aid the choice of the experimental conditions for in vitro tests derived from animals. The findings from these tests would then be compared with the effects of the same chemical in selected human cell preparations. Where the findings in the animal derived and human derived in vitro preparations are very similar and modelling of exposure and mode of action information indicates that the data is relevant to likely human exposure the information may be deemed sufficient for assessment of the risks to humans. Where the findings from animal derived in vitro preparations and from humans differ then additional in vivo studies in the animal species from which the in vitro preparation was derived is likely to be required to establish the relationship between the two and why the effects in the animal and human in vitro preparations. In the absence of a sound explanation default factors would have to be employed as at present.

Conclusions

There is enormous potential for *in vitro* tests to replace *in vivo* ones. In vitro preparations of varying degrees of sophistication are likely to have a role in all three tiers. Initially their principal use will be primarily at the tier 1 level but ultimately it may be possible for all tier 2 tests to be *in vitro*/*in silico*. However, in order to realise this, a number of major challenges need to be met

In principle there is no ideal *in vitro* model but each of them could be potentially able to address a specific toxicological question, pending the complete knowledge of their performance. The use of in vitro preparations derived from human organs would remove on level of uncertainty in establishing the effects of a chemical in man. However this is only the case if the in vitro preparation reflects the behaviour of the cells/tissues in vivo. A major barrier to the use of *in vitro* studies for risk assessment purposes is the lack of models viable for sufficiently prolonged time, reflecting adequately *in vivo* kinetics. This would be a specific need for tier 2 testing, in which more sophisticated *in vitro* preparations should be selected. In addition *in vitro* biokinetics data providing the actual level of cell exposure producing *in vitro* observed effects should be used to improve the dose-response curve with the aid of PBPK and PBPD modelling.

At present this is not achievable except for freshly derived tissue. In addition, cultures of single cell type are probably not the answer to this issue, and 3D models, mimicking the interrelationships between different cells will represent the future of *in vitro* research and testing. *In vitro* preparations of sigle specific cell types will nonetheless play a crucial role in the elucidation of modes of action and/or grouping of chemicals (Tier 2 and 3). The same issue applies to the use of animal derived tissues at the present time and therefore this problem is a major priority for the use of *in vitro* preparations as a replacement for *in vivo* ones.

The other major barrier to the dependence on *in vitro* studies for risk assessment purposes is the lack of viable models that reflect adequately *in vivo* kinetics. *In vitro* preparations will nonetheless play a crucial role in the elucidation of modes of action. The major problem with *in vitro* assays is the lack of knowledge about the link between any observed *in vitro* alteration and *in vivo* adversity: solving this issue it will be possible to translate specific alteration (a marker for a specific toxicological pathway') into adverse effects *in vivo* with potential role in the development of organ pathologies. In tier 3, modelling integrating in vitro and *in vivo* data will be play an important role.

5.6.4. "Omics" technologies

Introduction and current use

"Omics" technologies cover genomics, proteomics and metabolomics. The applied techniques are intended to give an overview of all changes in the specific area and try to correlate profiles or pattern of changes with biological effects. They contribute to understanding modes of action.

Genomics is a discipline in genetics that studies the genes and gene expression of organisms. The field covers intensive efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping efforts. The field also includes studies of intragenomic phenomena such as heterosis, epistasis, pleiotropy and other interactions between loci and alleles within the genome. In contrast, the investigation of the roles and functions of single genes is a primary focus of molecular biology or genetics and is a common topic of modern medical and biological research. Research of single genes does not fall into the definition of genomics unless the aim of this genetic, pathway, and functional information analysis is to elucidate its effect on, place in, and response to the entire genome's networks.

Toxicogenomics, which makes use of powerful DNA microarray technologies and measures the expression of thousands of genes simultaneously, has the potential to revolutionize toxicology. It has been used as a tool to elucidate mechanisms and to predict toxicity, including early toxicity screening. Predictive toxicology relies mainly on class prediction, whose methods are based on the assumption that gene expression profiles of known toxins from representative toxicological classes (reference compounds) can predict the toxicological effects of an unknown compound based on similarities between these gene expression profiles.

Proteomics analyse changes in protein expression. The proteome can be described as the total set of proteins produced by a cell, tissue or whole organism. Proteomics can be defined as a set of techniques aiming at identifying the proteome. The separation of complex protein mixtures is generally performed by two-dimensional gel-electrophoresis (2DG) and the subsequent analysis of isolated protein spots by mass spectrometry. Often, it is not the specific proteome that is analysed but the difference between the "normal" and the "exposed" or "diseased" proteome. The comparison of spots from different samples (e.g. healthy vs. diseased), with two-dimensional difference gel-electrophoresis (2D-DIGE), allows the detection of these differences in protein expression. The two dimensions in the separation are based on two significant different characteristics of the proteins. The first step is often based on isoelectric focusing, using strips with a fixed pH gradient, separating proteins according to their isoelectric point. In the second step, the proteins are separated on the basis of their mass, by SDS-PAGE. Then, individual spots on the gel are visualised, extracted and prepared for mass spectrometric analysis for identification. Sample preparation still remains the most crucial step preceding a proteomic analysis and can be complicated by the presence of salts or highly abundant proteins that may mask the presence of proteins present in small amounts. Commercially available tools can help overcome these hurdles and allow the detection of potentially interesting proteins. 2D-electrophoresis generally visualizes proteins between 20-200kDa in mass. Peptides (<20kDa) and small proteins are generally overlooked by conventional gel electrophoresis. This led to the development of separation methods based on liquid chromatography and introduced a new field of research called "peptidomics". To date, most studies compare healthy vs. diseased status. Progress in the field should enable shifting the focus towards proteins, as indicators of early responses to exposure or treatment. Together with proteomics, peptidomics already contributed to enlarge our knowledge of biological processes and, supported by sophisticated bioinformatics tools, to the discovery of new diagnostic and therapeutic targets.

Metabolomics is the characterization of metabolite profiles in blood or urine and the influence of the administration of toxic agents on these profiles. Metabolomics usually apply chemometric techniques such as ^{1}H-NMR or mass spectrometry and a mathematical transformation of the obtained data for pattern recognition. The modified patterns may be used as such to characterize a toxic response and thus may be used as biomarkers of toxic effects. ^{1}H-NMR-based metabonomics have been widely applied to characterize changes in kidney function by analysing urine after administration of nephrotoxic xenobiotics. The results may give indications regarding localisation of injury in specific segments of the kidney. Metabonomics may also been used to characterize specific aspects of liver toxicity or general changes in biofluid composition associated with disease progression. Metabolomics can be considered as a novel method for characterization of toxicity and be exploited to develop new biomarkers. In addition, support for mode-of-action or hypothesis generated studies can be performed based on metabolomics (Griffin, 2003).

Cytomics: In contrast to most "omics" techniques in which the molecular complexity of cells/tissues/organisms is investigated bottom-up (from genes to biomolecules, and organelles to cells, tissues and organs), cytomics uses a top-down strategy, assuming that the cell and not genes – or biomolecules - are the functional units of an organism. Moreover, comparing the molecular cell phenotypes of exposed vs non-exposed cells does not depend on detailed (a priori) knowledge of the effects (mechanisms) which makes cytomics a suitable tool in screening of unknown substances (Valet 2005).

Future potential

In order to realise the potential of omics, it is critical to ensure that the emphasis in using these techniques is on how changes in an omics profile varies according to:

- Duration, level and route of exposure to the chemical;
- Characteristics of the exposed organism/cell/tissue;

- Observed adverse effects in the same test system.

Omics is likely to increase greatly both the sensitivity and the amount of information produced in a test system. One consequence of this is likely to be the identification of changes below (perhaps well below) the currently recognised thresholds for adverse effects of many chemicals. In interpreting such changes, a distinction will need to be made between:

- True adverse effects that the previous methodology failed to detect;
- Changes which reflect a physiological (normal adaptive) response to a stressor which might be considered to be a sign of health of the test system;
- Changes that are not understood and require further investigation.

The integration of "omics", biomarkers and high content imaging for early prediction of toxicities *in vitro* holds a lot of promises. When toxins interact with cells and tissues they disturb the concentrations and fluxes of endogenous metabolites in key intermediary cellular metabolic pathways. In an attempt to maintain homeostasis and metabolic control, cells vary and equilibrate the compositions of their intra- and extra-cellular fluids. In more severe toxicity states, cell death leads to more dramatic biochemical changes due to loss of homeostasis and metabolite leakage from damaged cells. Whatever the severity of the toxic event, the subsequent alteration(s) in cellular biofluid composition are specific of the toxicity type. The use of a combined NMR and MS expert system approach allows to explore systematically the relationships between biofluid composition and toxicity and to generate novel combinations of safety biomarkers. The approach of characterizing the metabolic profile of a specific cell, tissue or biofluid has been termed "metabol(n)omics" by analogy with genomics and proteomics. ^{1}HNMR and MS-based spectroscopy's are well suited to the study of toxic events, as multi-component analyses on biological materials can be made simultaneously. The complementary role of NMR and MS spectroscopy in analytical toxicology is thus essentially one of biochemical exploration, i.e., determining the range of biochemical perturbations caused by exposure to a toxin and whether these are biologically significant.

Profiling methods mainly based on "omics" and high-content imaging as well as other endpoints capturing deregulation of essential cellular processes, will deliver biomarkers and cluster modelling data to be used for integration in the hazard assessment data sets for further risk assessment. Improved knowledge on the modes of action will be obtained for some model compounds. When sufficiently sensitive, metabonomics-based biomarkers may be used in a low-dose range to give a more precise characterisation of dose-response. They may also serve as additional biomarkers.

In the immediate future, data interpretation will be a real challenge.

Conclusions

Genomics is likely to play an increasing role in tier 1 assessment, but, along with proteomics and metabolomics, will also be applied in both tier 2 and tier 3. However, application of "omics" requires a strict quality control and a clear relation of "omics"-endpoints/profiles to adverse effects in animal models or in humans.

5.6.5. "Quantifying histological changes

Histology has provided the main tool for assessing adverse outcomes of chronic exposure of animals in vivo to stressors from the outset. This requires the sacrifice of the animals. The range of dyes used is generally very limited and are used to identify general changes in proteins and nucleic acids primarily. Histopathology is generally based on whole organ evaluation with a description of the lesions present and semi-quantitative estimation of the quantity rather than a real measurement. Specific dyes and the availability of (monoclonal)antibodies has improved detection of more specific lesions i.g. plaques formation in brain tissue. Histomorphometric analyis can be done on specific lesions for

more quantitative information. In some cases electron microscopy is used to enable morphological changes in individual cell types to be examined.

To realise the objectives of a largely or entirely in vitro approach to characterising the hazards arising from a particular exposure to a specific stressor, it will be necessary to:

Reflect the histological changes that occur in vivo to morphological changes in cell/tissue systems in vitro;

Translate the histological changes into related biochemical changes in order that earlier identification of adverse effects can be identified and understanding of modes of action advanced;

Establish techniques for the detection of early lesions in live animals.

A number of techniques may contribute to achieving the desired advances including quantitative histochemistry, whole body imaging and immunocytochemistry.

5.6.6. High-content image analysis

Introduction and current use

Acute toxicities are often related to cell necrosis that can be measured by blunt cytotoxicity endpoints. Sub-acute to chronic toxicities, however, are often related to effects at cellular level that disturb cell metabolism and/or cell structure without resulting in overt cell death, resulting in tissue alterations like local accumulation/deposition of proteins or chemicals. High content imaging (HCI) can elucidate mechanisms of toxicities and better predict more subtle toxic effects that are specifically induced after repeated dosing. HCI is based on immunocytochemistry that so far has been limited by the extremely labor intensive analysis and the subjective bias linked to the individual observer. By combining multi-channel fluorescence microscopy with automated data acquisition and powerful automated image analysis, HCI allows unbiased analysis of fluorescence microscope based endpoints. Using nuclear staining as an indicator for a cell, the HCI image analysis can report fluorescent image parameters on the level of the single cells. Different from flow cytometry, HCI also delivers information on morphology, movements, subcellular localization, co-localization, patterns and distances.

Also for classical stainings now sophisticated software has become available that allows digitalization of the tissue sections that can be further anlyzed automatically by so-called histology pattern recognition. This technique also allows for whole tissue sections to be quantitatively analyzed so a more complete overview is obtained.

For *in vitro* toxicity, profiling HCI is adaptable to any throughput that may be needed and allows addressing an array of relevant toxicity pathways within the same experiment.

Future potential

Multiple endpoints will be integrated at the level of single cells; gating and analysis of subpopulations will be used to determine toxicity mechanisms and population effects. Single endpoints as well as relevant combinations will be applied for the quantification and classification of toxicity with a scoring matrix and "cellomics" (not explained previously) data will be used for an integrated analysis together with the results of other methods. The integrated approach will deliver a very detailed view on the effects induced by each reference toxin. The integrated analysis may also show redundancies and will allow the selection of the most economic and meaningful set of assays to detect and characterize subacute to chronic toxicities. Established assays for HCI analysis on the

Cellomics Array Scan cover broadly relevant subcellular toxicity endpoints[12]. It is also likely to make an important contribution to the elucidation of modes of action because of its ability to link biochemical to quantitative morphological changes

The ability to digitize entire tissue specimens on slides and subsequently perform morphometric analysis on the images is suggested to be valuable in the rapid and consistent measurement of tissue features and biomarkers for pharmaceutical research and development.

Conclusions

The development of image analysis tools makes it possible for large scale evaluation of series of tissue sections. However, the quality of the staining used is of utmost importance when using these techniques.

5.6.7. Quantitative histochemistry

Introduction and Current use

Quantitative histochemistry enables the changes in proteins and nucleic acids within a cell type to be quantified and enzyme activities to be measured. Quantitative histochemistry has been used to investigate cellular changes arising in various diseases for several decades. However partly because of the specialist equipment required and partly because very few toxicological investigations have been conducted this potentially valuable technique has not so far found a place in the characterisation of hazards arising from particular stressors.

Future potential

Recent developments as described above in image analysis will also benefit the quantitative evaluation of histopathological lesions. Quantitative histochemistry has the potential to become an important tool both in the validation of *in vitro* methods as reflecting the *in vivo* situation and in the development of understanding of modes of action.

Conclusions

Quantitative histochemistry is unlikely to become a routine tool for hazard characterisation. However it is anticipated to be a valuable aid in ensuring that *in vivo* effects of a stressor are replicated *in vitro*.

C Tissue microarrays

Tissue microarrays (TMA) is a form of condensed histopathology in which cells and tissue are presented in a miniature multiplex platform for analysis (Takikita et al. 2007). It allows the sampling of multitudes of specimens of tissue to be analysed at one time. Most often formalin-fixed tissue specimens are used for evaluation although the technique can also be used on frozen tissues. TMA can be used for all methodologies that can be applied to a tissue section (Takikita et al., 2007), although immunohistochemistry remains the primary use. The collection and data interpretation is still performed by reviewing the slide under a microscope but the use of automated instruments is possible. Originally TMA was used for confirmation of data from gene expressions in microarrays for biomarkers, but is also used for DNA copy number analysis, in situ hybridization, cell line analysis, proteomics, and high throughput analysis of antibodies (Takikita et al., 2007).

[12] Straube F. (2005) The Bright Future of High Content Imaging in Drug Discovery and Development. Eur Pharmaceut Rev; in press.

Future potential: It can be expected that the use of TMA will increase depending on the development of new molecular markers. TMA can help to identify the validity of these markers and thus maybe useful in hazard identification as well. It is also suggested that it may be used for personalised medicine (Takikita et al., 2007).

In conclusion, he development of TMA has enabled high-throughput pathology that may be especially valuable for screening for biomarkers. The technique allows for the collection of a vast amount of data on specific biomarkers that allows evaluation of their value for both diagnosis and as prognostic factors. Such evaluations may also be usefull in the hazard identification for safety evaluation and risk assessment.

5.6.8. Whole body/organ scanning

Introduction and current use

Modern imaging technologies make it possible to investigate biological processes on various levels, from the molecule up to the whole body. For whole body or organ scanning nuclear magnetic resonance imaging (MRI) offer high soft tissue contrast and the opportunity to identify the development of lesions such as tumors in live animals. General disadvantages with MRI include operational complexity and many technical variables involved which may influence and compromise the reproducibility of the investigations. To date, MRI has been used sparingly in toxicology. The reasons for this include the lack of commercial availability of small NMR equipment, the problems of restraining the animals during the examination and the lack of background data on imaging in laboratory animals.

Other relevant technologies for whole body and organ scanning include computed tomography (CT) scans and positron emission tomography (PET) imaging. The former has the advantage of offering high reproducibility and can contemporarily evaluate soft tissues, lung parenchyma and bone. However, the use of X-rays can provide limits to extent of use also in animal studies. Micro-PET systems for animal studies are increasingly used as a non-invasive tool which can measure substances such as pharmaceuticals or toxic agents in target organs. For both these technologies, the availability of instruments, and their costs, limit their current use in toxicological studies.

Future potential

Advances in the development of small powerful magnets are likely to result in quite extensive use of MRI for identifying disease in domestic animals. It is unlikely that such techniques could play a part in routine toxicity testing. However they may be useful to study the development of a specific lesion over time and might contribute to the establishment of early detection methods for particular categories of adverse effect. The same is valid also for CT scans and PET imaging.

Conclusions

Whole body imaging is unlikely to be used widely for hazard characterisation. Nonetheless it will provide an important tool for specific investigations.

5.6.9. Implanted sensors

Current use

Rapid advances in microelectronics have enabled a limited use of devices to be implanted that can potentially monitor levels of some drugs in the body. However to date the applications are theoretical rather than practical.

Future potential

In principle such devices could be used to assess exposure to a number of chemicals simultaneously by sending data to a remote receiver or to one carried by the individual being monitored.

As well as the practical issues of the implant and removal of implant processes there are important ethical issues that will need to be resolved.

The principal barrier is the development of appropriate devices that measure chemicals that are important to the assurance of health of the individuals concerned.

Conclusions

Although in principle this is an exciting development for the measurement of internal exposure there are serious ethical and practical constraints that will need to be addressed.

5.6.10. Mode of action studies

Introduction and current use

Mode of action may be defined as a sequence of key events and processes starting with the interaction of a chemical with one or more cell components and proceeding through a succession of biological events to the frank expression of disease. Modes of action should be defined at the molecular/biochemical level.

The value of mode of action studies is shown by our current approach to the early identification of carcinogenic chemicals. Genotoxicity tests for carcinogens are based on mode of action research, which linked initial mutations caused by chemicals to the subject development of cancer. Mode of action information on genotoxicity has also been used to interpret the relevance of findings from life time carcinogenicity studies in laboratory animals.

The mode of action for a stressor that causes toxicity can be considered to consist of three parts:

- Initial (primary) interactions between the stressor and biological components. This has been termed the critical molecular interaction event by the OECD.
- Intermediary (secondary) stage(s) as a consequence of the initial interaction(s); these may include irreversible steps and/or additional effects on key metabolic pathways (termed adverse outcome pathways or toxicity pathways by OECD and NAS) resulting from prolonged exposure to the stressor. This will also require an understanding of cell-response networks.
- Late (tertiary) stage(s) in which evident effects on health are manifested Termed critical organ and tissue responses by OECD).

The links between these stages must be understood if the relevance of data from animal or *in vitro* models to man is to be considered.

In the development of medicines and pesticides most commonly the approach is based on an understanding of desired modes of action.

For industrial chemicals, the main reasons for conducting a mode of action study were to explain differences between species or to explain why the findings in a particular species are not relevant to man (e.g. peroxisome proliferation in the liver of rodents caused by

phthalates). There is a good understanding of the modes of action of a number of highly toxic chemicals, such as dioxins.

There have been many recent publications highlighting the importance of mode of action studies including (Keller et al., 2012), (Boobis, 2010; Carmichael et al., 2011; Julien et al., 2009). A number have also set out to define modes of action.

Future potential

All of the above methods can be brought to bear on the identification of modes of action. Mode of action studies are likely to use a variety of *in vitro* models and apply 'omics' along with a range of other methods to measure specific biological components and events. From a scientific perspective, understanding modes of action along with some confirmatory data indicating relevance to man is the soundest basis for risk assessment. The ultimate aim must be to ensure that modes of action provide the core data base for risk assessments. Thus a key issue in developing the future paradigm for risk assessment is to improve understanding of modes of action. It is vital that mode of action studies are the focal point for future research in toxicology. It will need to draw heavily on developments in biological and medical sciences on causes and exacerbating factors for human diseases and hence facilitation of a continuing dialogue between the relevant disciplines needs to be established

Mode of action information would prove invaluable for other elements of the paradigm development including:

- The design, development and use of *in vitro* tests

- New strategies for read across;

- The development of biomarkers;

- The identification of groups in the population likely to be susceptible to particular groups of chemicals;

- The development of a new classification of chemicals and the ability to predict likely interactions between chemicals in the body.

-The assessment of the potential effects of exposure to mixtures

Reliable information on modes of action will provide a sound scientific basis for risk assessment. Unfortunately, the identification of the relevant modes of action for a particular chemical is expensive and time consuming. Nonetheless the benefits greatly outweigh the disadvantages.

Mode of action studies will enable key data gaps and information that would be of value in the further risk assessment of a chemical, such as dose-response relationships and recognition of potentially susceptible subgroups, for example, life-stage considerations. To facilitate progress in the development of mode of action studies it is recommended that a repository of accepted MOAs and associated guidance is established concerning appropriate data to support specific MOAs for critical effects. This would facilitate categorization of chemicals and allow predictions of toxicity outcomes by read-across.

The mode of action studies will provide many challenges including:

-to identify critical initial interactions from other early interactions

-to identify critical pathways

-to distinguish between physiological and adverse responses.

Criteria need to be established to guide researchers in addressing these challenging issues. These criteria should include: the strength, consistency and specificity of the association, dose response concordance, temporal comparability, biological plausibility, a comparison with alternative modes of action considered and uncertainties, and data gaps.

Lack of funding is the main barrier to progress despite the fact that funding for the development of *in vitro* tests has been rather generous. The development of useful *in vitro* tests would be much more soundly based and more acceptable for risk assessment purposes if mode of action information was available.

Conclusions

Mode of action studies must become the central plank of a future risk assessment along with reliable and relevant exposure assessment. In considering promotion of the above technologies attention should be given to how they might facilitate advances in understanding modes of action and how the technologies could benefit from knowledge of modes of action.

5.7.Effect of combination of stressors

Future risk assessment will have to take increasing account of exposure to both chemicals and non-chemical stressors. The definition of "non-chemical stressor" in the context of risk assessment is very difficult but is generally defined as physical, e.g. radiation, noise, climatic conditions, biological encompassing pathogenic agents, e.g. HBV and AFB in the induction of liver cancer or physiological and social stressors e.g. deficiencies in the quality of a person's environment or resources to affect people's health. These non-chemical stressors have the potential to either directly affect the health independently of chemical stressors or indirectly by modulating the responses to chemical exposures. It is important to design studies that allow for a more defined measurement of the relative contribution of chemical and non-chemical stressors to disease. To add to the complexity the exposure for the chemical, biological, physical and physiological stressors show large temporal and spatial variation in the life time of individuals.

Susceptibility to the effects of chemical, physical and biological stressors may be affected by psychological stress. It is normally chronic stress, rather than acute stress, that is hypothesizes to increase individuals' susceptibility to pollutions via altered glucocorticoid responsitivity. Risk assessment currently often focuses on the risk of single or complex mixtures of chemicals and does not adequately incorporate non chemical stressors and the important aspect of vulnerability into the assessment process. The relevance of non-chemical stressors in risk assessment is indicated by the large disparities in health risk across population groups with the focus on vulnerability (Sexton, 2011). There are several examples from the experimental and epidemiological literature that chemicals and non-chemical stressors are inducing the same effect, e.g. air pollution and stress both induce the blood pressure, noise and organic solvent impairs the hearing by different modes of action. Such interactions need to be assessed.

Epidemiological investigations are the best available tool for assessing the impact in man of multiple exposures to stressors. In the case of social stressors, it is important to differentiate between individual vs community stressors. Most of the epidemiology-based research involves establishing statistical associations with specific social stress, but this association most likely does not prove causality. It remains important to understand the biological basis in order to establish the casual relationship. Many social stressors are difficult to measurable but different approaches has been taken by WHO (urban heart) and Cumulative Environmental Hazard Inequality Index (CEHII) (Su, 2009).

There is growing evidence that stress may influence one or more of the physiological pathways as certain chemical toxicants (Wright, 2009) thus biomarkers of effects are likely to be an important tool for assessing exposure to multiple stressors. Biomarkers of effect of some non-chemical and chemical stressors may be mediated by molecular signaling among the same biochemical pathways.

Surrogate environmental species might also be useful for identifying specific impacts of multiple stressors. A role for *in vitro* biological preparations placed in selected environmental settings may also be foreseen.

5.8.Data, databases and data repositories

Introduction and current use

Currently sourcing data for individual risk assessments depends largely on de novo searches using search engines such as Medline, Toxline and Pubmed. Data not published in the scientific and medical literature is not accessible in this way.

Ready access to properly validated and up to date data bases is essential to:

- Avoid unnecessary duplication of experiments;
- Provide the basis for TTC-like tier 1 assessments;
- Facilitate test design;
- Inform the choice of test conditions (see *in silico* methods);
- Develop computer based tools (e.g. chemical space estimates and SAR and QSAR tools);
- Aid mode of action studies.

REACH is in the process of generating the largest database on chemicals in history. For each registered substances, REACH collects the following key information:

- Main physical-chemical properties
- Environmental fate and pathways
- Toxicity and ecotoxicological information with summaries of each study
- Identified uses described through a combination of five descriptors

A number of commercial databases currently exist (see appendix) particularly on data following oral exposure of laboratory rats. The databases on *in vitro* test findings are less well developed and those of the effects of chemicals in man are so far of very limited value.Key issues are the quality of the databases, their accessibility for risk assessment purposes and the nature of the expertise required for their use.

The quality of any data used in risk assessment is of utmost importance in relation to usefulness and reliability. This applies to data on physicochemical properties, biological activity, and exposure assessment.

A number of factors can affect quality ranging from entry errors to the most frequently encountered issue of data being derived from tests with inadequate design or quality control. In view of this, preference should be given to data produced according to standardised procedures (e.g. OECD or US EPA method).

The reliability and robustness of *in silico* predictive models is also intrinsically dependent on the quality of the data used in building and validating the model. This includes data on chemical identity, chemical structure, isomeric form, chemical descriptors, biological activity data, statistical algorithms used in building the model, and the degree to which the model was tested and validated. For *in silico* model development, biological test data from good laboratory practice (GLP) is often sufficiently reliable. The scarcity of good quality toxicity data for a sufficient number of related compounds is often the limiting factor in the development of robust and reliable *in silico* models. A data quality algorithm has been proposed by Malazizi et al (2006).

As discussed above one of the crucial foundations for the development of a new paradigm for risk assessment is the availability of comprehensive databases. This is essential for

the validation of new models and methods and the development of QSARs. Five categories of databases are needed:

1. Human exposure data;
2. Human effects data from exposure to individual chemicals;
3. Data on the adverse effects of chemicals in animal models;
4. *In vitro* findings;
5. Metabolism and other kinetic data.

There is also a need for access to data repositories in biology, medicine and chemistry because utilisation of progress in these fields is needed to achieve the desired advances in the risk assessment of chemicals.

Expert systems need to be developed to enable these databases to be searched intelligently and efficiently.

In addition to the obvious resourcing requirements to achieve this, a major barrier is the large amount of existing data that is currently not accessible because of commercial confidentially issues. Means must be found to address these.

Consideration is also needed on how to identify and utilise the information being obtained through scientific advances in systems biology, synthetic biology, regenerative medicine and tissue engineering) for the development of test systems, to aid mode of action studies and to better understand the health implication of certain hazards.

Conclusions

The establishment of validated, readily accessible up to date databases must provide the foundation for the future risk assessment paradigm. The principal barriers to progress are not scientific ones and stakeholder dialogue is necessary as a first step to overcome these.

5.9.Acceptability criteria for the use of methods

New methodology should only be considered where it provides clear advantages for risk assessment. It is therefore important to identify the criteria for acceptance of new tests/assessment procedures. The most important is that it is relevant to effects on human health and has a strong scientific basis. Other criteria that should be applied are:

- Reproducibility and sensitivity
- Ethical acceptability
- Reflects exposure conditions likely to be experienced by humans
- Low-moderate cost and potential for improved throughput

5.10. Priorities for change

It is very timely to review current approaches to risk assessment. There are major external pressures to change. It is also appropriate from a scientific viewpoint. Recent major advances in the understanding of biological processes, along with the increasing availability of rapid screening and data processing tools provides new opportunities and challenges.

To improve exposure assessment, the first priority is to improve current approaches to exposure assessment. A reliable exposure assessment is essential to prioritise chemicals for hazard assessment.

-The development of *in vitro* testing strategies to determine relevant doses for toxicity testing for risk assessment purposes is another priority. Adler et al. indicate that kinetic info is crucial to understand 'internal exposure'. Usually when exposure is cited, the external exposure is implicitly the reference. More efforts should be given to the measurement of internal exposure: in this sense, if there is no possibility to have any systemic dose, the process could stop. It could be useful to introduce the concept of internal TTC and/or PBPK modelling. This priority has overlapping aspects with the other listed ones, but expands their area of applicability.

-A third priority is to develop improved understanding of modes of action of toxicologically important chemicals. This will provide an essential scientifically justified base for characterising threshold for adverse effects and identifying vulnerable population groups. It would also enable a sound basis for read across, a relevant framework for the grouping of chemicals and for the risk assessment of mixtures.

5.11. Future strategy for exposure assessment

The new methodology needs to be able to estimate reliably aggregate and cumulative exposure and variability in human exposure. There are several drivers for an improvement in exposure assessment:

- The increasing recognition that exposure assessment is the weakest aspect of most risk assessments and that for animal use reducing and other purposes more reliable exposure assessments are vital. The use of TTC depends critically on reliable exposure assessment.
- Legal and other requirements (e.g. REACH) requires exposure data for submissions. This will place much greater emphasis on the provision of exposure data than was previously the case. Moreover the increasing acceptance of TTC as a means of limiting unnecessary testing will also give a much greater weighting to reliable exposure assessments.
- The increasing availability of new techniques for measurement of chemicals and their biological effects, e.g. exposomes.

In order to accomplish the required paradigm shift to exposure driven risk assessments major advances are needed in exposure assessment. Without such progress an exposure driven approach to risk assessment cannot be widely implemented. The objectives for exposure assessments for humans must be the development of robust predictive models, coupling external and internal exposure calculation. The need for predictive approaches arises from the requirement of dealing with a large number of chemicals in a complex variety of situations. While the current approach to exposure prediction are in general qualitative or, at best, semi-quantitative, the goal of such approaches should be the implementation of source-exposure- outcome paradigm based upon an integrated (coupled) external (environmental fate, occupational exposure and food uptake) and internal (toxicokinetic, such as PBPK models) dynamic exposure model and bioindicators of effect. With such tools at hand, it would be possible to obtain quantitative results of exposure for humans, taking into account the spatial and temporal variability of chemical contact and interaction depending on life cycle (age-variant exposure), lifestyle, occupational situation and environmental exposure variations. This would allow linking multiple exposure routes of parent compounds and metabolites, possibly including in the simulation of the exposure concentration chemicals of similar mode of action, as to obtain a total exposure simulation. The integrated modelling approach, being quantitative, would allow to fine tune the threshold of tolerable usage and emission of a chemical (including metabolites and same mode of action compounds) in a complex exposure situation

New approaches to improve the quality of exposure assessments include improved conventional measurements and modeling based upon refined technologies, sensors including mobile phones and portable computerized devices.

A staged approach to the assessment of exposure from individual chemicals is recommended in which priorities for further work on individual chemicals are determined particularly by the estimated human exposure (nature, routes, levels, duration).

The initial assessment (tier 1) may be based on very conservative modeling of external exposure. This should take account of lifetime exposure to the chemical from all sources and exposure to chemicals with potentially comparable modes of action. Tier 1 might also incorporate in vitro and/or in silico data on the likely absorption through the relevant exposure routes. This exposure assessment should be based upon the worst case situation considering all routes of exposure. The exposure levels can then be compared with a benchmark of exposure acceptability such as the TTC. Improved models for exposure assessment are needed to ensure that tier 1 is a robust process. For the development and the validation of such models, reliable and relatively broad based exposure data bases are a prerequisite. Models requiring development include ones to predict total exposure to a chemical from all sources and models to identify total exposure from chemicals and other stressors with comparable modes of action.

For chemicals for which exposure is deemed to exceed this acceptable level, a more realistic and comprehensive evaluation of external exposure and absorption potential will be needed (tier 2). For this purpose, the ability to refer to a comprehensive database on exposures would again be valuable. Computer simulation of potential metabolites may also indicate the need for some metabolism investigations at least in vitro.

The findings from these studies may, depending on the estimates of concern, lead to a tier 3 assessment in which PBPK modeling and external exposure models as well as more sophisticated measurements would be needed, including information on usage as well as descriptors of the exposed population.

Tier 3 exposure assessment should be based upon biomarker data taking advantage of the new molecular technologies and the mode of action. The usage of exposomes e.g. bioindicators of adverse effects, is one such approach.

The tiered approach needs to be mirrored by a tiered approach for managing the uncertainties in the exposure assessment. Tiers range from a worst case or conservative assessment, through one or more refined deterministic assessments to fully probabilistic assessments. At all stages, additional information received, e.g. monitoring data, dose-response data, information on uses, can improve the assessment and reduce, or sometimes increase, the uncertainty. In the first tier, the conservative approach, the uncertainties are treated intrinsically by using worst case exposure situations, assumptions and default values. One should realise that it is not always easy to analyse whether these exposure situations are really worst case.

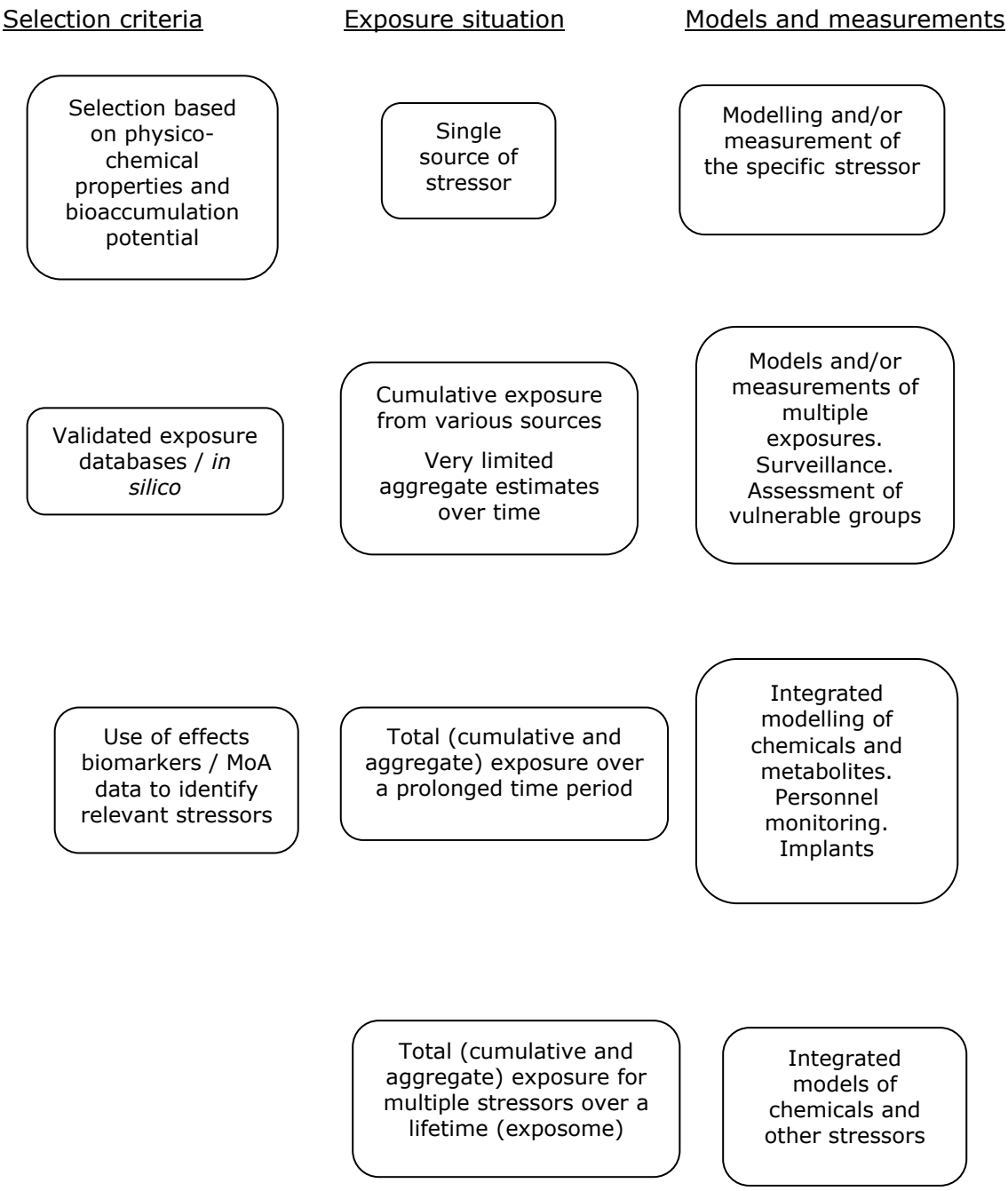
A critical aspect of the future development of exposure assessment is feedback once a chemical is marketed so that estimated exposures and actual exposures can be compared. It is noted that the development of new monitoring techniques eg personal monitors will also make post marketing surveillance of the air borne exposure to chemicals of concern/interest to be carried out in a straightforward and relatively low cost manner.

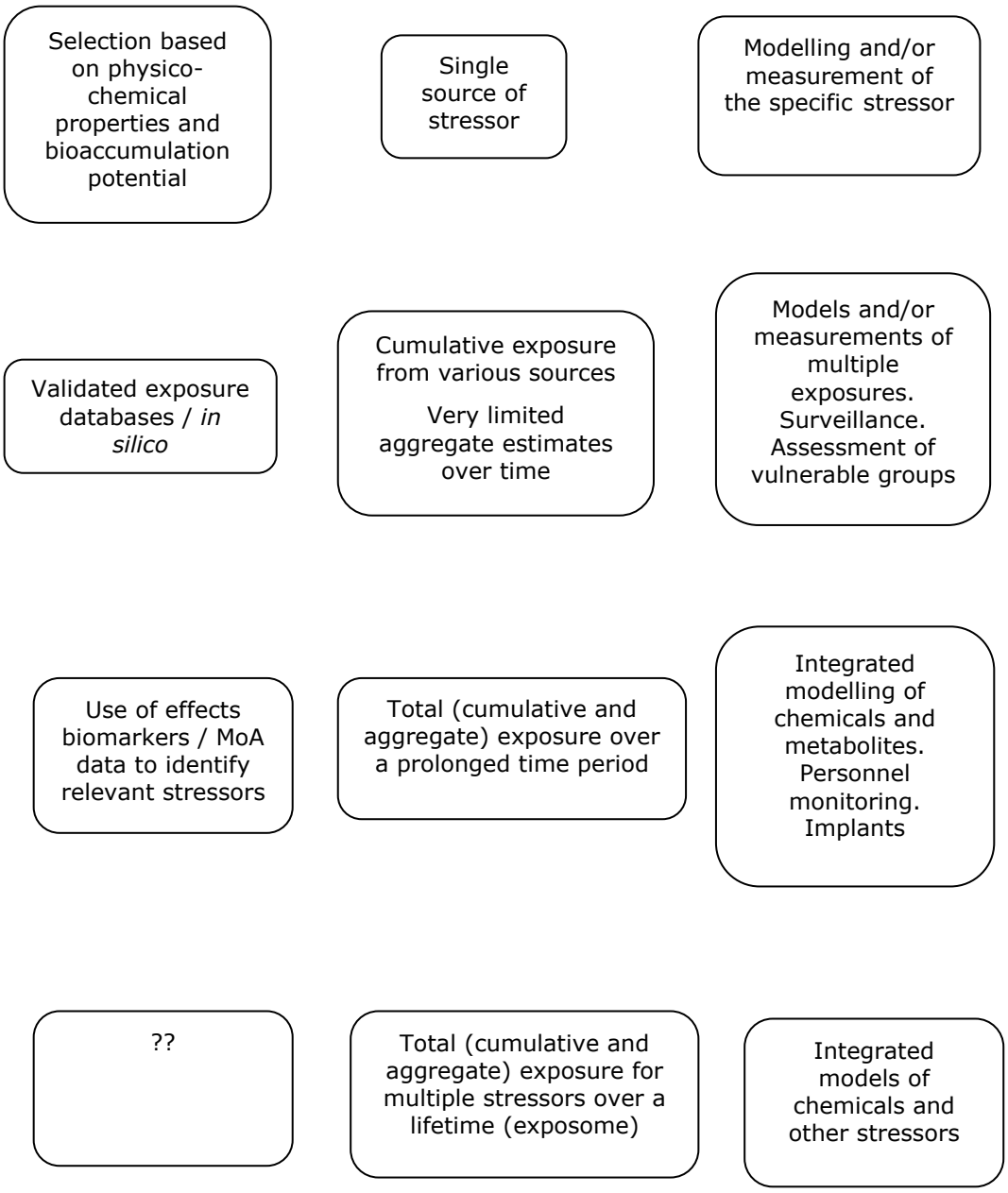
As noted above exposure assessment is typically the weakest point in risk assessments. In order to remedy this major investment in model development and low cost high throughput measurement devices is required. Also very important is the development of training for exposure assessors.

A roadmap for exposure assessment

It is important that the critical steps to achieve the future exposure paradigm are identified. Three strands need to be considered: the selection of the exposure measurements or modelling, the nature and route of the likely exposure and the actual measurement and modelling techniques. This road map is set out in Figure 6.

Figure 6: A road map for the development of an exposure assessment methodology (NB the vertical dimension indicates landmarks with time)





The vertical dimension in this road map indicates major changes that are likely to be achieved progressively over a period of several decades. There are three issues that need to be considered:

- What is the focus of the measurements/modelling(exposure situation)
- The models and/measurements that may be used
- The information available to inform the exposure assessment (selection criteria)

In using this road map the starting point is the selection parameter. Currently the principal criterion in selecting a testing procedure (in addition to regulatory requirements)is the physiochemical properties of the chemical under investigation. This information should in the future be used to inform the choice of model and/or measurement technique(s) to be used.

5.12. Future strategy for hazard assessment

Particular scientific concerns are:

- Hazard identification and characterisation depend on the assumption that humans and laboratory rodents respond in a very similar way to each stressor;
- Understanding how each stressor causes its adverse effects is given low priority;
- Most risk assessments are qualitative or semi quantitative at best;
- Risk assessments are often expressed in terms which are not easy to apply in practice by risk managers and other stakeholders.

It is essential that each of these issues is addressed.

Other key drivers to change the way hazard characterisation is conducted are:

- Public and political opposition to the use of animals in toxicity testing resulting in a high emphasis on *in vitro* and *in silico* test development;
- High costs and slow time to carry out the currently required battery of tests with potential consequences for negative impacts on innovation;
- Major developments in measurement technologies enabling potential new testing procedures (see following sections);
- Advances in the understanding of the sequence of changes underlying many diseases; this greatly facilitates a mode of action approach to the assessment of the hazards from chemicals. The recent introduction of the overarching concept of the toxosome (toxome) to embrace the life time effects on health due to exposure to chemicals.

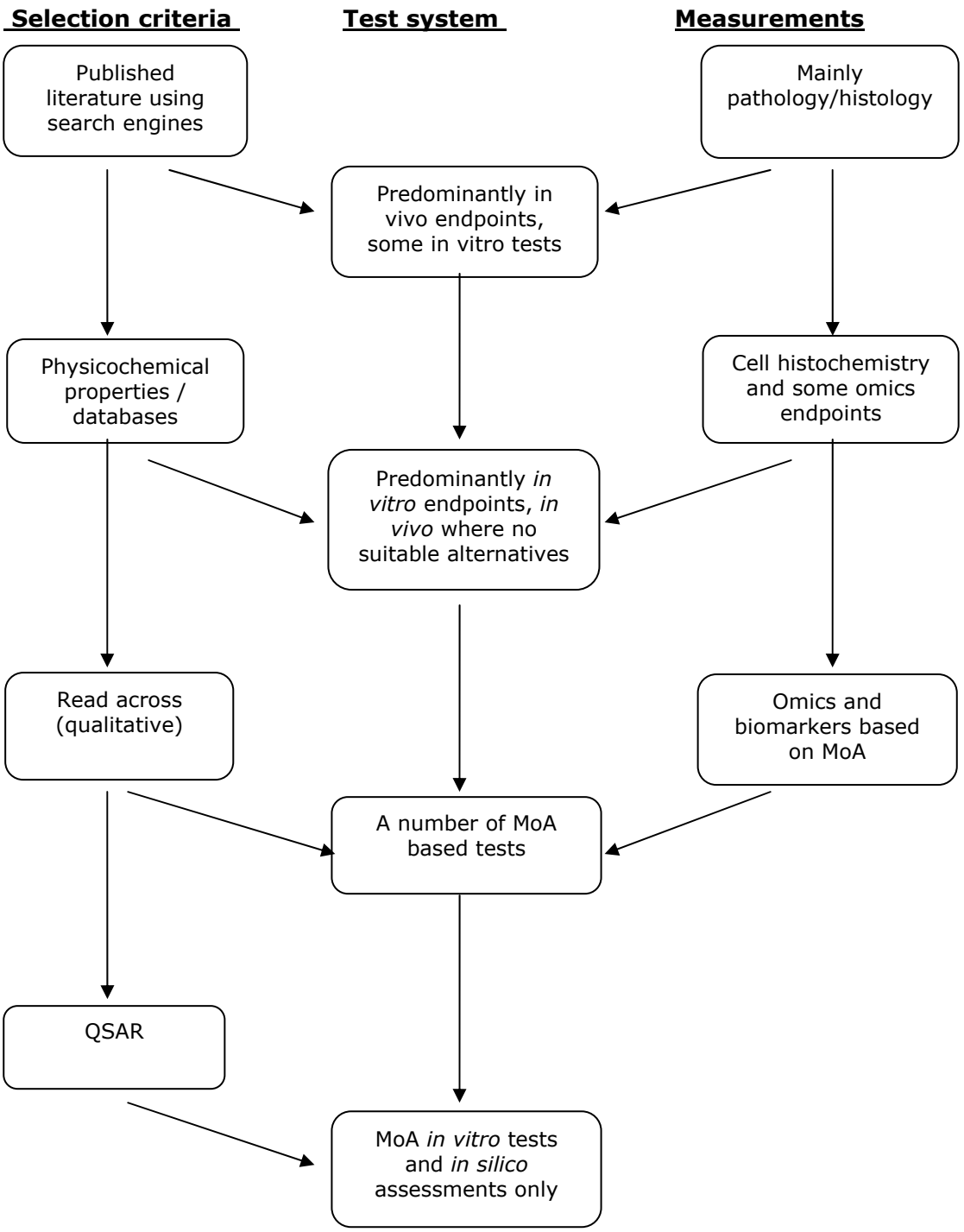
A road map for hazard assessment

Having identified a future strategy, it is also important to identify the critical steps along the road to its achievement. These are set out in Figure 7. The three strands to the road map are:

1. Selection criteria ie the progressive use of existing information to inform the selection of both tests and test conditions
2. The nature of the test systems available
3. The types of measurements available.

The start of the road map is inevitably the current approach with the ultimate objective of characterisation based on a thorough understanding of modes of action. It is difficult to set dates by which each step will be achieved since each depends on progress in research and in acceptance of each change.

Figure 7: A road map for the development of hazard characterisation methodology



It is recognised that changes to the classical paradigm for risk assessment will require specific training of data generators, risk assessors and risk managers The paradigm identifies that a progressive evolution in the methodology will need to occur. It is vital that those concerned with the risk assessment process both understand the methodology involved and the way it is applied for risk assessment purposes. Various levels of training are envisaged ranging from basic course that address mainly the principles involved to advanced training to ensure that the data generated by the new methodologies are critically evaluated and appropriately applied.

5.13. Proposal for a testing strategy

The initial assessment (tier 1) should be conducted for all stressors where a significant potential exposure of human is deemed as possible, as illustrated in Figure 8.

Tier 1.

Before embarking on any hazard assessment the databases should be searched for any useful information on the stressor under examination.

Tier 1 tests should be based on simple tests for specific effects, the selection of which is informed by physicochemical properties and *in silico* information in particular structure-activity relationships. Test systems must have a suitable 'drug' metabolism capability. False positive effects are much more acceptable than false negatives because an unrecognised false negative could result in a conclusion that there are no hazardous properties of concern. On the other hand, a false positive result could cause the unjustified stop in the development of a 'useful' chemical. For the immediate future a number of these tests will be classical endpoint-based, but eventually mode of action based tests are anticipated to be predominant. Tier 1 should include tests for:

- Cytotoxicity (different organs, Acute and repeated)
- Irritancy/sensitization
- Genotoxicity
- Neurotoxicity
- Endocrine effects
- Production of reactive metabolites *in silico* and *in vitro*

For each endpoint relevant reference standards should be employed.

At the present time a wholly *in vitro* methodology for tier 1 is not possible and limited *in vivo* tests are therefore needed. Based on the state of current knowledge it would be appropriate to rely largely on existing *in vitro* test for genotoxicity, irritancy and cytotoxicity *in vitro* tests for tier 1 will be likely available in the near future. Tests for various other endpoints are under development and could be introduced subsequently. In tier 1, where ecotoxicity data exists this should also be considered.

Tier 2.

The principle aim of tier 2 tests is to characterise properly those effects identified as of possible concern in tier 1 tests and the determination of the dose response relationships for each such effect. Consequently considerable flexibility will be needed in the selection of testing protocols in tier 2. A critical element of such tests is to ensure that the exposure conditions used include those that are reflective of exposures likely to occur to humans in practice and of response sensitivities that are reflective of human tissues *in vivo.* For this purpose at Tier 2 comparisons of rodent based and human based tests may be appropriate. In tier 2 more sophisticated *in vitro* preparations, including those involving prolonged exposure of the preparations to the stressor, will be required that in tier 1 and multiple measurements employed. The selection of these should draw on the *in silico* data.

Ultimately it may be possible for all tier 2 tests to be in vitro/in silico. However currently in vitro methodology may be valuable as a follow up to specific aspects of a particular effect (i.e. identification of MoA), but are not sufficiently advanced yet to play a substantial role in tier 2 testing. Consequently reliance for the near future must be on in vivo methods. For many stressors sufficient information on the adverse effects and the exposure conditions required to manifest them may be gained in tier 2 that further assessment is not required. If significant effects are anticipated at likely exposure levels,

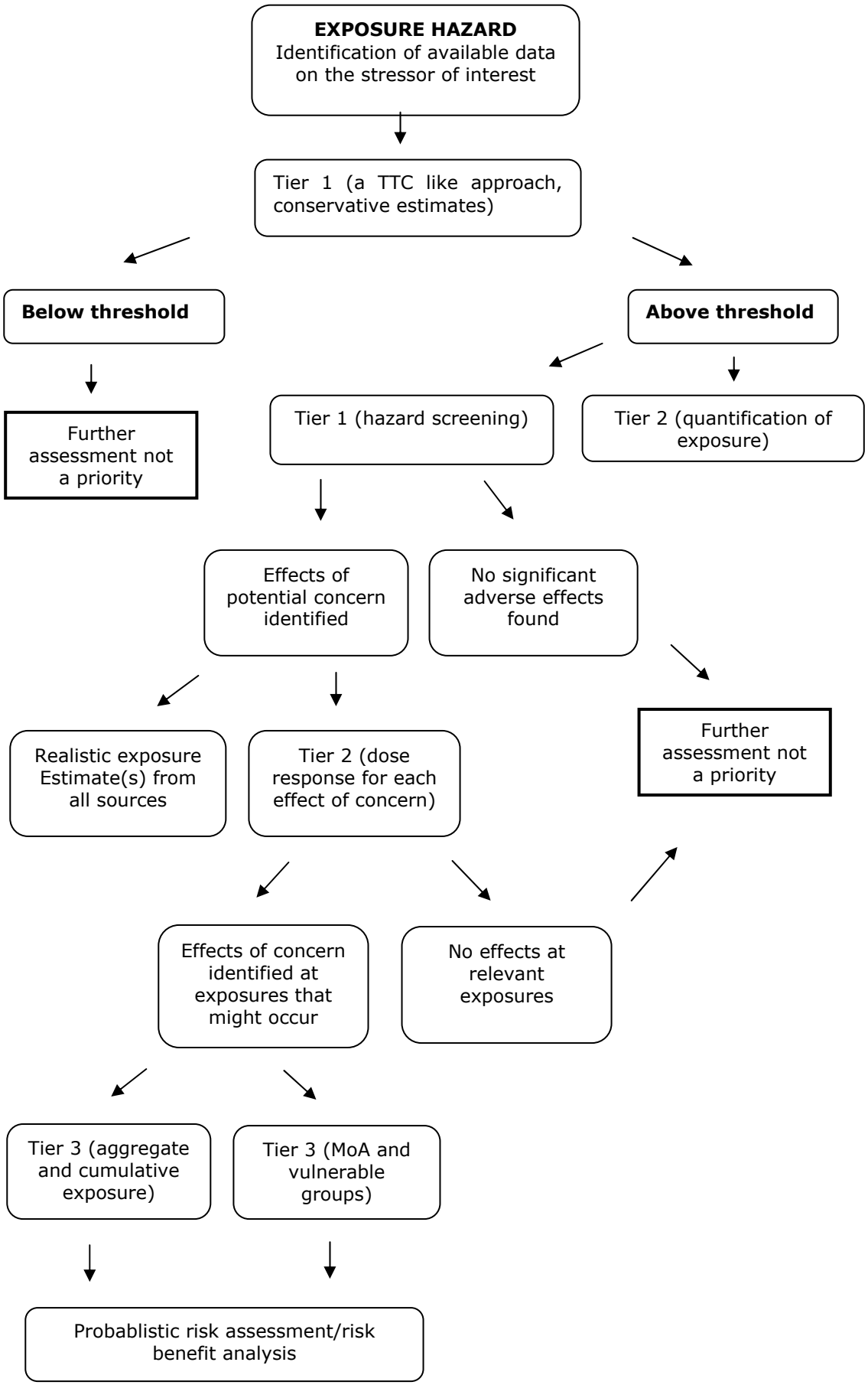
and alternatives to the use of the stressor under these exposure conditions are not identifiable, then tier 3 assessment may be required.

Tier 3.

Tier 3 should be focussed on probabilistic risks assessment along with the development of an understanding of the mode/mechanisms by which a particular stressor, identified as of potential concern based on tier 1 and tier 2 findings, produces the specific effects of concern. Based on such information potential vulnerable groups of the exposed human population may be identified. In this tier, formal tests are not likely to be appropriate rather in vitro preparations should be selected to enable the modes/mechanisms of action and the factors that influence this. In tier 3 in vitro techniques are anticipated play an important role in both the short and long terms alongside in vivo investigations.

> Risk assessments have generally been of a deterministic rather than probabilistic nature. This has focussed attention on the likelihood of a specific threshold value for the effects of a chemical in man, the assumption being that no significant adverse effects will occur below this threshold. Often, this is with little or no specific knowledge of human variability in the expected response of individuals to each chemical, although default factors are used to account for these potential differences. If it is not obvious from this analysis that risks are adequately controlled, the deterministic risk assessment can be refined in subsequent tiers with an increasingly thorough analysis of uncertainties. The uncertainty assessment at the first refinement stage should list and classify all relevant quantifiable and non-quantifiable uncertainties as well as the influence a specific entry has on the risk and the effect of all uncertainties combined. The third tier, the probabilistic approach, concentrates on the assessment of the quantifiable uncertainties. The PRA gives quantitative insight into the range of possible outcomes and the degree of cumulated conservatism in the exposure assessment. It forces experts to reveal the nature and extent of their judgment on types of uncertainty and distributions, whereas sensitivity analysis can reveal the relative impact of uncertainties in parameters on the final result and where improvements are most time- and cost efficient. In the risk characterisation, the combined effect of all identified uncertainties, quantifyable as well as non-quantifyable, in both the exposure assessment and the effects assessment should be evaluated carefully.

Figure 8: Application of the tiered strategy.



6. OVERALL CONCLUSIONS

The essential practical purpose of risk assessment is to identify whether action is needed to control/reduce/prevent exposure to one or more chemicals through a soundly based scientific procedure for identifying and characterising the risks (along with any significant uncertainties in the analysis). The shift from a simple to more complex, dynamic approach is a general trend that applies to both ecological and human health risk assessment. The aim must be to achieve a very high level of accuracy in the estimation of all significant adverse effects that may occur in man and/or the environment from likely exposure conditions to a stressor or a combination of stressors.

This will require methods that enable the diversity of responses that might occur to be identified and the characterisation of the conditions and science based explanations involved.

The Scientific Committees are aware that the most of the proposals described in this discussion paper are not realistically suitable for amending, in the short term, risk assessment procedures for regulatory purposes. However, this discussion paper highligts needs and priorities for research in order to get the objective of higher precision, accuracy and transparency in risk assessment for protecting human and environmental healh.

6.1. Ecological risk assessment

The Scientific Committees consider that there are large margins for improvement in ecological risk assessment beyond procedural details. It is recognised that there is the need for a change in the philosophy of ERA, moving from the reductionist methods of the past (on which many European regulatory tools have been successfully based) to a more holistic approach capable of increasing the ecological realism of the assessment and of explaining and predicting the actual effects that may occur on structure and functions of complex natural ecosystems. This need, that may be assumed as a new paradigm in ecological risk assessment, has been already acknowledged by the ecotoxicological scientific community and, in the last few years, many new tools have been developed or are under development in order to provide some suitable answers.

This general need for more ecologically based assessment can be applied in a different way for different regulatory tools. On one side, existing regulations aimed at the registration of chemicals at the European level (e.g., REACH, pesticide/biocide directive) can be improved by increasing ecological realism while maintaining applicability at wide geographical scales. This could be achieved by developing European ecological scenarios for effect assessment, comparable to the scenarios developed by FOCUS for exposure assessment. The issues listed below should be related to these scenarios. On the other side, regulation aimed at protecting specific ecosystems, e.g. the Water Framework Directive, will require more site-specific approaches accounting for the complexity and variability of biological communities.

The need for improvement involves both the traditional components of ERA (exposure and effect assessment), but also introduces a new component, particularly relevant for site-specific risk assessment: the evaluation of the characteristics (sensitivity, vulnerability) of biological communities and ecosystems potentially exposed.

Several issues have been highlighted that may be relevant for developing more ecologically based risk assessmente approaches. A scheme for an improved risk assessment procedure is described in Figure 9. However, it is the opinion of the Scientific Committees that, for many of these issues, there is the need for more research to allow suitable application for practical purposes. In particular, in some cases, substantial efforts

must be made for transferring present and future knowledge from basic science to regulatory purposes.

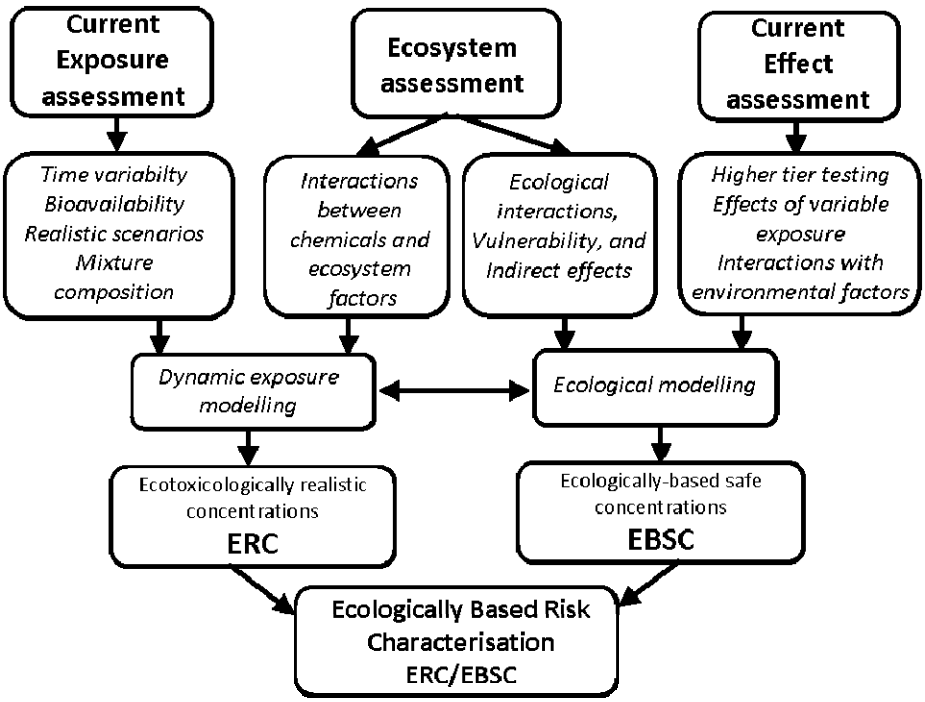


Figure 9. Scheme for a more ecologically realistic risk assessment procedure.

As for the traditional ecological risk assessment approach, a tiered procedure can be applied by refining any step of the assessment if the previous tiers indicate the possibility of an unacceptable risk. The complexity of this scheme underlines the difficulty of applying a hazard-based assessment in ecotoxicology adopting concepts comparable to the TTC applied for human risk assessment.

A road map can be proposed in order to suggest some priorities either for the possible application and use of available knowledge or for more substantial need for research to be developed. In these priorities, the problem of risk assessment for mixtures is not mentioned because the issue has been the objective of a specific opinion (SCHER, SCCS, SCENIHR, 2011).

6.1.1. Exposure assessment

The issues described below are listed in a sequence that largely corresponds to increasing challenges for immediate application and needs for further research.

Improving the practical usefulness of available experimental data. In this case, the problem could be solved with the development of precise rules and criteria for the use of monitoring data for assessing exposure to individual chemicals and mixtures. In particular data should be gathered at increased spatial and temporal resolution to cover the range of exposure situations of ecosystems. This should be done creating suitable databases, harmonizing existing and new data (such as GIS and remote sensing data) according to QA/QC rules. . Regarding physical-chemical properties, existing data and those provided by REACH should be integrated and quality validated. Extremely important could be the use of information that may derive from some European regulalatory tools, such as the Water Framework Directive. Regarding physical-chemical properties, the information that will be provided by REACH represents a powerful tool and

its rational use nedd to be implemented. For the armonisation of data and for actually improving their usefulness it is essential the development of detailed protocols for the description of required metadata, necessary to allow the use of experimental data in risk assessment. Finally, it would be relevant to provide data beyond REACH and WFD (or other European regulations). The development of international databases of global relevance should be strongly supported.

Accounting for bioavailability. For some chemical classes (metals and non polar organics) some tools for assessing bioavailability already exist and are often applied in ERA. More precise rule must be established for accounting for bioavailability. For other classes of chemicals, such as polar organics, there is the need for specific research.

Distribution and fate modelling. Even if many improvement are possible and have been highlighted in this document, modelling already represents the most important tool for exposure risk assessment in ERA. However, there is a need to develop models capable to predict time and space variable concentrations to account for realistic exposure situations, together with improved food web models, especially for terrestrial environments. Additionally, research is needed for developing models suitable for predicting the fate of polar and ionized chemicals and for nanomaterials. For these last substances, fully new approaches need to be developed, conceptually different from the traditional molecular-based models.

6.1.2. Effects assessment

More ecologically-based tools are particularly relevant for effects assessment but, in many cases, more knowledge and specific research is needed before they can be realistically applied in ERA for regulatory purposes. As above, issues are listed in a sequence indicating increasing research needs.

Higher tier and sub-individual testing. Higher tier testing (micro and mesocosms, SSD) is already used successfully in ERA. Research is needed for a more transparent and statistically based evaluation of the uncertainty.Sub-individual endpoints (omics, biomarkers, etc.), in contrast, are currently of little use in ERA. Research is needed to understand the actual meaning of sub-individual endpoints to understand effects on structure and functions of ecosystems.

Toxicokinetic/toxicodinamic (TK/TD) models. The improvement of these models, that represent a tool for describing effects of irregular exposure patterns, is a key priority for research. Indeed, variable (e.g. pulse) exposure is the most frequent situation in the real environment.

Ecologically relevant mechanisms. These issues, such as the assessment of ecosystem vulnerability, of indirect ecological effects, of interactions between chemical stressors and other environmental conditions, represent another important priority for research. In the last few years many efforts have been made on these topics. However the possibility of taking into account these mechanisms in a quantitative and transparent way for regulatory purposes is still far to be achieved.

Ecological predictive tools. Very promising tools exist to describe the behaviour of biological communities and ecosystems, such as trait-based assessment and ecological modelling. However, a big research effort is needed to transfer the knowledge from basic science to practical and regulatory purposes. In particular, ecological models represent the most logical tool for describing and predicting the behaviour of ecosystems under stress. In this sense, they represent the future of ERA and one of the most important priorities for research. They are already used occasionally, but their reliability and soundness for regulatory purposes need to be better proved and validated. Depending on the species and ecosystems of concern, and the research efforts, this can be achieved within 5-10 years.

6.2.Human-health risk assessment

The essential practical purpose of risk assessment is to identify whether action is needed to control/reduce/prevent exposure to one or more chemicals through a soundly based scientific procedure for identifying and characterising the risks (along with any significant uncertainties in the analysis). The alternatives to this approach for the management of chemicals (a hazard based approach or a precautionary approach), although having the benefits of simplicity from a regulatory standpoint, will inevitably result in actions that are illogical from a health protection standpoint and will inhibit innovation. It may also result in the approval of some stressors that pose a health threat to humans.

Over time toxicity tests have been increasingly standardised by the introduction of good laboratory practice and ICH or OECD test guidelines. Some *in vitro* tests, in particular for genotoxicity and topical effects have been added. Many of the tests in current use are written into legislative requirements for the approval of various types of products.

To address uncertainties due to the need for extrapolation when using data obtained in rats and mice to characterise effects that may occur in humans, conservative standard default values (also called assessment factors, uncertainty factors or default factors) have come into common use.

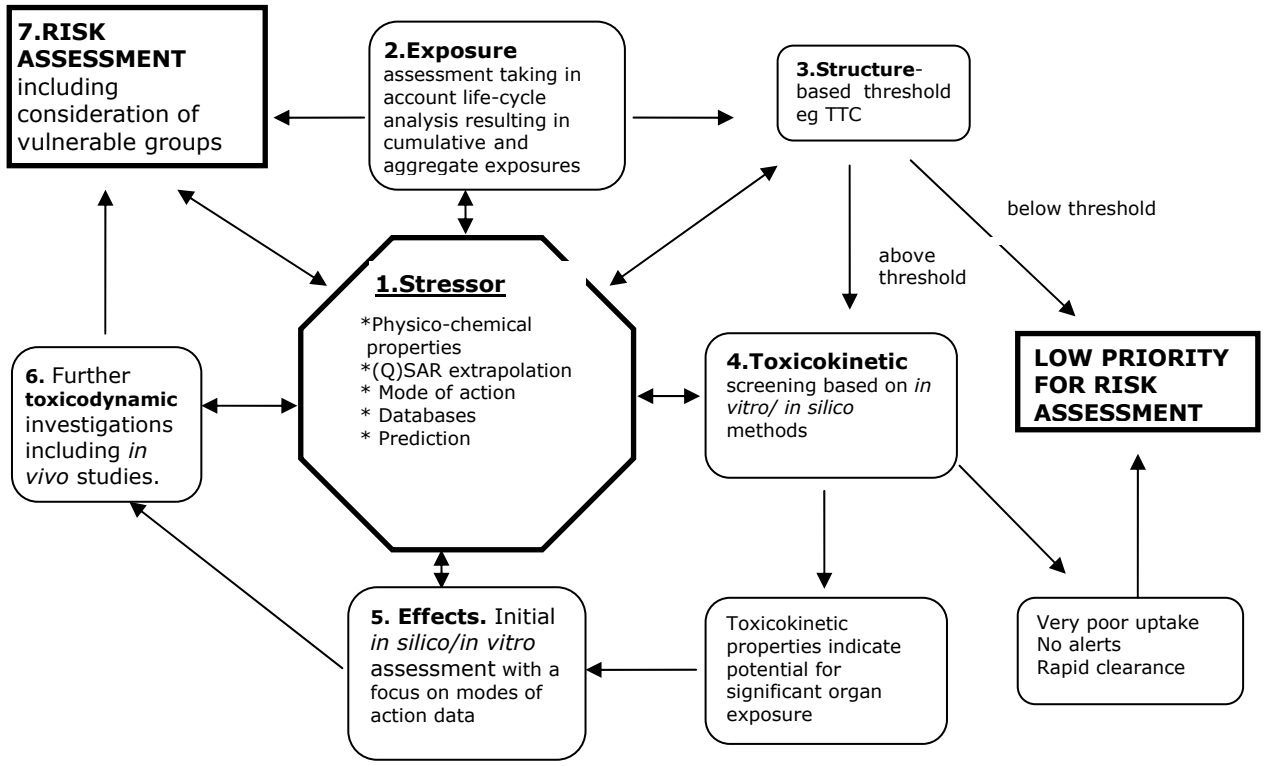
The aim of a risk assessment should be to achieve a very high level of accuracy in the estimation of all significant adverse effects that may occur in man from likely exposure conditions to a stressor or a combination of stressors. This requires methods that enable the diversity of responses (e.g. potential vulnerable groups) that might occur to be identified along with the characterisation of the conditions and science based explanations involved. Major advances in both the basic sciences and in medicine offer the opportunity to make major improvements in risk assessment procedures in the future. To achieve this, an important requirement is the better coordination of the necessary research and a willingness to draw on on-going work that traditionally is not associated with toxicology and exposure science.

There are good scientific and ethical reasons for this current review of the risk assessment process and for identifying new developments that would be likely to lead to substantial improvements. The changes that have been identified are evolutionary not revolutionary as they depend on further advances in a number of technologies. Nonetheless it is envisaged that the end result will be a radical departure from existing risk assessment procedures. The primary changes proposed may be characterised as follows:

- A paradigm shift from a hazard-driven process to one that is exposure-driven,
- A progressive reduction of tests using laboratory animals to be replaced by a mode-of-action framework.

This is reflected in the figure 10 shown below:

Figure 10 A dynamic model for future human risk assessment



The starting point is to consider what is known about the stressor based on its physicochemical properties and on information in the databases. This may enable some prediction of potential properties that are of concern and require specific investigation. The next step (step 2) is to estimate exposure based on life-cycle analysis. This could be a tiered process (see below). In step 3, the nature and levels of exposure are compared against structure-based thresholds for adverse effects (a TTC like approach) assuming the appropriate databases become available. At this stage a decision may be made that a particular stressor is not a priority for further assessment because the anticipated exposure levels are too low. However, to make this decision, a reconsideration of the data available on the stressor and the validity of the exposure estimate should be carried out. For stressors where further assessment is deemed necessary attention should be given to the likely toxicokinetic properties of the stressor (step 4). In particular whether it is likely to be adsorbed and whether, on absorption, it is expected to persist in body tissues or be rapidly cleared. This information should aid the design and conduct of hazard identification tests which is the central role of step 5. It is envisaged that in future step 5 may depend entirely on *in vitro* findings and *in silico* information. This is not feasible in the immediate future. Based on the findings in the first 5 steps, it may be concluded that there is sufficient information to decide that the stressor does not pose a risk judged to be significant and is therefore a low priority for further assessment. If this is not the case, a more thorough examination of the toxicodynamic properties should be carried out (step 6). This stage may be tiered as discussed above. These data, along with that from all the other steps, should then be used to conduct the final risk assessment.

6.2.1. Exposure assessment

The quantification of exposure, both in individuals and in populations, is a prerequisite for the quantification of risk. Reliable data on exposure are needed to assess the probability of adverse effects of the stressor and to recognize specific risk factors such as

occupation, life style, and social status. The dimensions of exposure include intensity, frequency, route, and duration; in addition, the nature, size, and makeup of the exposed population should be characterized.

Although a number of major advances in both chemical identification and quantitative analysis have been achieved, exposure assessment remains the weakest part of the majority of human risk assessments. There are a number of reasons for this:

- In the development of the current risk assessment methodology improvements in exposure assessment have been given lower priority.
- Human exposure assessments often rely on assumptions on consumer behaviour that may result in biases.
- Investigations have tended to focus on single sources of exposure rather than on multiple sources which is the more common exposure situation.
- Often exposure assessments have given insufficient attention to bio- and chemo-transformation.
- Human exposure is a highly variable parameter.

Advances in exposure assessment are crucial. The techniques that appear to be most promising to assess external exposure are:

Monitoring of external personal exposure

In order to define the exposure scenarios, a better understanding of lifetime activities would be needed. Developments should be directed to obtaining:

- Estimates of both typical and high exposures in different age groups and the factors that most influence this, i.e. use pattern;
- Information on trends in exposure over time to particular chemicals of 'concern', due to societal or behavioural changes.

The development of new monitoring techniques (e.g. personal monitors) will also make post marketing surveillance of exposure to air borne chemicals easier and cheaper. To assess airborne exposure to chemicals, especially in the workplace, a particularly desirable development would be the availability of low cost personal samplers to enable individual exposures to be assessed. The increasing availability of better absorbents and advances in technologies such as 'the laboratory on a chip' make this a realistic prospect. The main issues are to ensure proper prioritisation based on which groups of chemicals to measure and in which media. The availability of information provided within REACH would be essential for the prioritization and for the definition of appriopriate exposure scenarios. The main barrier will be the availability of low cost high throughput measuring devices able to measure accurately a wide range of chemicals and their transformation products.

Prediction of external exposure

There are a number of challenges for improving models of external exposure: one descends from the need of accounting for variability in space and time of environmental concentrations (including food). This would allow to better estimate human variability in exposure, especially at different stages of the life cycle. Other issues are related to the many uncertainties in the understanding of the behaviour of polar chemicals, nanomaterials, mixtures, as well as bioaccumulation in food of different origin. There is also a need for a harmonized approach in modelling strategies for different categories of chemicals, such as industial substances and plant protection products.

Modelling of exposure will only represent the external dose, but combined with PBPK this information can be transformed into the internal dose.

Determination of internal exposure

The techniques to estimate internal exposure depend on information of external exposure but also require improved knowledge on absorption, distribution, metabolism, and

excretion. It needs to be recognized that the development of *in vitro* systems requires appropriate methods to apply each chemical as well as uptake and metabolism systems that reflect those *in vivo*. Developments in PBPK modeling is likely to be an important tool by which the *in vitro* to *in vivo* interface can be improved.

Modelling integration

Exposure prediction should be implemented based upon an integrated (coupled) external (lifecycle analysis, occupational and consumer exposure) and internal (toxicokinetic, such as PBPK models) dynamic exposure model and biomarkers of exposure. The integrated modelling approach, being quantitative, would allow to finetune the threshold of tolerable usage and emission of a chemical (including metabolites and same mode of action compounds) in a complex exposure situation.

Use of a tiered approach

A tiered approach to the assessment of exposure to chemicals is recommended in which priorities for further work on chemicals are determined particularly by the estimated human exposure (nature, routes, levels, duration).

6.2.2. Hazard assessment

There is likely to be a continuing pressure to replace animal testing for risk assessment, labeling and classification by one or more of the following methods: non-testing methods, such as grouping and read-across, Thresholds of Toxicological Concern, exposure-based waiving, and computational methods (SARs, QSARs, biokinetic modelling), *in vitro* tests, and optimised *in vivo* tests such as the Extended One-Generation Reproductive Toxicity Test. Since most of such alternative methods cannot be used as stand alone, it will be necessary to integrate them into a so-called integrated or intelligent testing strategy (ITS) based on Weight-of-Evidence methods integrating several of the above mentioned independent sources of information and information on mode or mechanisms of action (Boobis et al., 2008; Martin et al., 2007). A shift is foreseen towards using more and more human data on biologically significant perturbations in key toxicity pathways, in such integrated testing strategies.

Key requirements for this are:

New *in vitro* methods

Prerequisites are:

- Establishment of *in vitro* preparations that preserve properties similar to their *in vivo* counterparts for prolonged periods of time;
- Means of reflecting *in vivo* toxicokinetics *in vitro*;
- Establishment of clear relationship between *in vitro* endpoints and adverse effects *in vivo*.

New endpoints

Sensitive measurement methods are needed to allow studies to be made at exposure levels that reflect likely human exposures. Omics is likely to play progressively a key role in identifying potential hazardous endpoints.

Mode of action

Modes of action identify the adverse outcome pathways that link exposure to a chemical to immediate or eventual outcomes. Although studies of the mode of action are a focal point in the development of medicines and pesticides, this is not yet the case for industrial chemicals. Mode of action studies must become the central point of a future risk assessment along with reliable and relevant exposure assessment. In considering promotion of the above technologies, attention should be given to how they might

facilitate advances in understanding modes of action and how the technologies could benefit from knowledge of modes of action. Mode of action information is also essential for the assessment/prediction of chemical interactions in mixtures.

A tiered approach

To enable the most effective use of resources and to limit the unnecessary use of animals a tiered approach to the assessment of hazards from exposure to individual stressors has been identified. Before conducting a hazard characterisation information should be sought on previous studies on the stressor under examination.

Databases

The availability of comprehensive, validated and up-to-date databases is the essential foundation for the development of the new paradigm. Of the various needs that have been identified above the most important are:

- Effects of various stressors in humans;
- Measurement/monitoring data on human exposure to various stressors;
- Extending the database that is a prerequisite for the TTC;
- The modes of action responsible for each type of adverse effect;
- Validation of SARs or QSARs and read-across approaches.

6.2.3. Risk characterization

Development and application of the paradigm will involve input from a new range of methods and tools. This will require a much greater dependence on scientific judgement in order to better assess the weight of evidence. For example it will be essential to distinguish between changes which should be deemed as normal physiological changes to a stressor and a response that should be considered as adverse. This has major implications for the training and range of research experience of future risk assessors.

6.3.Issues common to human-health and ecological risk assessment

6.3.1. Flexibility and transparency

Many regulatory instruments define current risk assessment procedures. Although this has advantages, it tends to reinforce a check-list approach to risk assessment and hampers the introduction of new methods as opposed to a more rational approach. For example, in a number of domains it reinforces the application of standard default (uncertainty) factors.

As new methods are developed their role in risk assessment needs to be identified. They should not just be considered simply as further tests that should be conducted. The move to a new risk assessment paradigm focussed on an 'intelligent' approach will require high transparency both in the data generation and in its analysis for risk assessment purposes. This will put a high emphasis on how the assays for hazard identification are selected and the resulting data are weighed. This is the subject of a memorandum of the SCENIHR (2012).

For human risk assessment, the development of the databases, *in vitro* and *in silico* techniques and understanding of modes of action is likely to enable a new scientifically sound approach to stressor classification. The current trend to a hazard-based classification for labelling and regulation makes little scientific sense.

For ecological risk assessment, the use of more ecologically-based approaches, some of them already frequently applied (e.g. mesocosms, SSD, field and semi-field studies),

would require a sound assessment of uncertainties. Statistical tools for a quantitative assessment of uncertainty would substantially improve the transparency of the assessment.

6.3.2. Uncertainty analysis

Uncertainty in risk assessment in the general sense is defined by IPCS (2004) as "imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration". There are different types of uncertainty, some quantifiable and others not, some reducible and others not. Besides uncertainty due to lack of knowledge, variability adds to overall uncertainty. Ignoring uncertainty may lead to incomplete risk assessments, poor decision-making and poor risk communication. Uncertainty is inherent to each of the four steps in risk assessment. Risk assessors and risk managers have to take uncertainty into account and they should realise that 'high quality (in policy-related science) does not require the elimination of uncertainty, but rather its effective management' (Funtowicz and Ravetz, 1990). The strength of evidence is inversely related to the degree of uncertainty. The degree to which characterisation of uncertainty (and variability) is needed will depend on the risk assessment and risk management contexts as determined in the questions asked ie problem formulation (see SCENIHR 2012).

Expert committees deal with uncertainty by applying judgement based on accumulated knowledge of the subject. Sometimes committees evaluating identical data reach different conclusions as a result of differences in judgement by different experts and differences in terminology. In spite of significant progress in the area of uncertainty analysis, uncertainty and variability is often not, or insufficiently, addressed. At best uncertainty is qualitatively or semi-quantitatively addressed. Most of the currently conducted risk assessments are deterministic rather than probabilistic. In deterministic regulatory risk assessment standard default values are often used to allow for identifiable uncertainties. Where probabilistic risk assessments are conducted worst case scenarios are often built in and in order to identify the uncertainty these conservative assumptions need to be properly characterized.
PRA can use all information about quantifiable variability and uncertainty in both the exposure and the effects assessment and forces experts to reveal the nature and extent of their judgment, e.g., on types of uncertainty, distributions, the shape of the dose-response curve and the nature of the critical effect. Sensitivity analysis is able to reveal the relative impact of uncertainties in parameters on the final result and can reveal where the risk assessment can be improved in the most time- and cost-efficient manner and whether it is necessary and achievable to reduce the uncertainty further.
The approach for uncertainty analysis in both exposure and hazard assessment needs the following:

- A clear separation needs to be made between uncertainty due to lack of knowledge and variability to be able to answer different risk questions.
- It should be made very clear which uncertainties due to lack of knowledge and variabilities are included in the assessment and which not.
- Overall, statistical tools for a quantitative assessment of uncertainty would substantially improve the transparency of the assessment.
- Non-quantifiable uncertainties such as poor data quality, model uncertainty or subjective choices cannot easily be addressed in the probabilistic approach and should be dealt with qualitatively, using standardised terminology.

These requirements will have to be met to support the envisaged more exposure driven risk assessment which is at the same time more based on non-testing methods and integrated testing strategies. *The uncertainty in non-testing methods* needs to be captured in terms of statistical indices of predictivity and reliability weighting factors. Formal decision analysis methods can support the application of testing strategies based on a combination of testing and non-testing information. *With regard to*

exposure, predictive approaches should be improved by developing integrated models capable to predict time and space variable concentrations and doses to reflect the ecological and human variability of conditions and more realistically predict ecosystem and human health exposure. *With regard to hazard assessment*, tiered approaches for uncertainty analysis need to be developed to be able to capture quantitatively the uncertainty and variability in dose-response assessment, adjustments and extrapolations.

6.3.3. Training needs

To ensure good progress towards the new paradigm for risk assessment recruitment, training and opportunities to gain relevant experience are essential. This will entail substantial changes in the requisite skills base. It is also necessary to recognise that the assumed reliance on the new methodology proposed for both hazard and exposure assessment will initially at least require a much greater emphasis on the use of judgement by risk assessors. In addition to the expertise in toxicology, ecotoxicology and exposure science, generation, validation and interpretation of data would require higher-level expertise in biostatistics and modelling. Consequently, teams of very experienced risk assessors will be needed. It appears very unlikely that the current availability of risk assessors will be sufficient to meet these demands. Training in each area would require to be at a number of levels and will need to include ability to make judgements across different disciplines.

6.3.4. Harmonising risk assessment procedures

Harmonisation of risk assessment procedures between domains of stressor use and between nations is highly desirable. It would reduce unnecessary use of experimental animals and other resources and would avoid differences in the outcomes of risk assessments due to variations in the risk assessment procedures between sectors and between countries. Of course, harmonisation is much more difficult to attain once data requirements and risk assessment procedures have been written into regulatory instruments. An important issue to be resolved is the inability of the current fragmented research approaches to deliver meaningful mature input for regulatory purposes. The actual barriers are the understanding of the problem and possibilities to propose various solutions, and to deliver on them, accessibility of tools, and awareness for their proper use (with understanding of scope, advantages and limitations).

The development of new technologies provides an opportunity to achieve harmonisation across sectors and countries on the conduct of the new methods and the interpretation of the findings before they become embodied in legislation. Because of the pressures to reduce animal use and to patent new methods and other factors this will not be easy.

It is recommended that the Commission Services give particular attention to ways in which a dialogue on the issues associated with the development and implementation of the new methodology can be established and maintained. This might be based initially on the road maps set out above.

One option would be to support the establishment of an independent, professional, multidisciplinary Europe wide, academy of risk assessors that would work with the US National Academy of Sciences and similar bodies in other nations involved in risk assessment advice.

6.4.Recommendations and research needs

6.4.1. Databases

The availability of comprehensive, validated and up to date data bases is the essential foundation for the development of the new paradigm. Of the various needs that have been identified above the most important are:

- Collection of data on the effects of various stressors in humans. There is much unpublished data on the adverse effects of various drugs in man. In addition this data is continually being recorded in the workplace, in poisons centres, in volunteer tests as well, as from epidemiological studies.
- Collection of measurement/monitoring data on human exposure to various stressors. This includes chemical physical and biological stressors.
- Extending the data base that is a prerequisite for the TTC (tier 1) application. It should be noted that this is a critical step in reducing the number of stressors requiring further investigation.

In addition there is a need to find means of linking progress in other areas of science eg systems biology and medicine to advancing the scientific base for risk assessment.

For ecological risk assessment, at present some extensive databases exists on ecotoxicological data (e.g. ECOTOX, US EPA 2012) providing the results of toxicological tests for a wide number of chemicals on different aquatic and terrestrial organisms. However, for the development of more ecologically-based approaches, there is the need for a deeper knowledge on bio-ecological data on structure and processes at different levels of hierarchical organisation (from species to ecosystem). For example, the lack of information on complex traits (metabolic, physiologic, behavioural, etc.) is the major obstacle for the development of trait-based risk assessment. Additionally, environmental exposure data should be collected (including their metadata) and made openly available, especially for chemical mixtures and metabolites, for a variety of environmental scenarios. There is a large body of physical-chemical properties data which requires verification and validation. The systematic collection and armonisation at international, over-European, level of these data would substantially improve the development of experimental and predictive approaches, as well as the understanding of the different modes of action of the same chemical on different living organisms.

6.4.2. New models for effects prediction

There is a very large body of research going on in many countries with the aim of replacing in vivo tests by in vitro ones. Progress has been disappointing slow. There are two major technical challenges that need to be focussed on:

- The encapsulation of in vivo exposure conditions in in vitro tests.
- The preservation of the all the normal biochemical and physiological functions of cells for periods of several weeks following removal from an animal/human. This is essential for the replacement of chronic tests in animals by *in vitro* tests.

In silico methods

The development of in silico tests is inevitably bound up with the availability of suitable databases.

The use of in silico predictive methods offers a rapid, cost-effective and ethical alternative to testing toxicity of chemical substances in animals. The availability of reliable chemical property/effect databases, powerful data mining algorithms, and enormous computational power in the past decade have all led to the development of more versatile and reliable computational tools for the assessment of chemical toxicity. These tools are mainly based on structure-activity relationship (SAR), quantitative structure-activity relationship (QSAR), or read-across between analogous chemicals.

The models based on (Q)SAR are mathematical descriptions of biological activity of a group of chemical compounds in terms of one or more of their physicochemical properties. The origins of (Q)SAR go back to the observation that, biological activity in a closely related series of chemicals varied according to steric, electronic, and hydrophobic properties of the series, which could be expressed mathematically.

QSARs generally take the form of a linear equation:

Biological Activity = Const + (C_1,P_1) + (C_2,P_2) + (C_3,P_3) +….,

where the parameters P_1 through P_n are computed for each molecule in the series and the coefficients C_1 through C_n are calculated by fitting variations in the parameters and the physicochemical property or the biological activity. However, a quantitative model could be of non-linear nature as well.

SARs on the other hand describe qualitative relationships between chemical structure and a property or biological activity. In its simplest form a SAR takes the form of a 'structural alert', which represents a distinctive feature in a molecule which bears a relationship with a property or biological activity.

Expert systems combine the different in silico approaches to predict bioactivity of a chemical substance from its structure. These may comprise decision-trees based on rules, structural alerts, and/or nested (Q)SARs. For example, the use of two Experts systems TOPKAT® (Toxicity Prediction by Komputer-Assisted Technology; Accelrys Inc) and Derek for Windows® (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd) for the assessment of heat-derived toxicants in food has been described by Cotterill et al (2008). A more recent account of the available (Q)SAR tools and Expert systems for toxicity assessment of chemical substances in silico has been provided by Price and Chaudhry (2011).

Over the years, the development of (Q)SARs has progressed from one or two simple properties (e.g. molecular size, hydrophobicity) of a related series of molecules and their activity at the molecular level (e.g. enzyme inhibition, or receptor binding), to more diverse mix of chemical structures and complex toxicological endpoints in whole organism. This brings into consideration a number of other factors that can modulate toxicity, such as penetration through biological membrane barriers, biokinetics, possible metabolic transformations, etc. The increased complexity in the biological response at the whole organism level has lead to the use of a much wider range of descriptors of molecular properties to encode such complexities. This in turn has necessitated consideration of more complex mathematical models based on non-linear algorithms and soft-computing techniques, such as fuzzy systems, probabilistic methods, and artificial neural networks (ANNs) to decipher relational patterns in large, imprecise, and complex datasets.

The selection of appropriate algorithm is therefore a critical step in (Q)SAR development. For numerical and continuous biological data (e.g. LD50), the use of multiple regression based methods would generally be feasible. If however the biological response is discrete, (e.g. active/inactive, or inactive/weak/moderate/strong), then choice may include decision trees, neural networks, support vector classification, or clustering. The choice of different statistical algorithms for QSAR building has been reviewed by Chaudhry et al. (2007).

Once developed, (Q)SAR models are tested rigorously for robustness and predictivity, as well as for the 'applicability domain' for each model, which needs to be clearly defined. The applicability domain of a (Q)SAR model is the response and chemical structure space in which the model makes predictions with a given reliability (Netzeva et al., 2005). A fully tested and validated (Q)SAR model would generally yield good predictive assessment of the toxicity of an untested chemical as long as the compound is within the domain of the model's prediction space. Thus each model has certain limitations when used against a varied range of chemicals from different classes. Such limitations can be overcome by the use of a combination of different (Q)SAR models, Expert systems, and read-across approaches. This way sufficient 'weight of evidence' can be generated to achieve a reliable assessment.

A variety of (Q)SAR models is currently available for predicting complex biological phenomena, including a number of toxicity endpoints of regulatory significance. Some of these models have been developed for regulatory use, in accordance with to the stringent

quality criteria and validations principles laid down by the OECD. Examples include QSAR models developed under the EU projects DEMETRA (www.demetra-tox.net/index.php) and CAESAR (www.caesar-project.eu/). Another example is the OECD QSAR ToolBox, which is a versatile suite of programs that can predict a range of endpoints for chemicals based on read-across, structural similarity, or QSAR, using a substantial set of high quality databases.

The use of *in silico* methods (QSARs) in ecotoxicology is a suitable tool for the prediction of tradidional single-species toxicity and the development of user-friendly tools is strongly supported. Moreover, the development of *in silico* approaches may represent a powerful tool for trait-based models for predicting the responses (sensitivity, recovery capability, vulnerability) of populations to stress factors. Some promising examples of the application of chemometric methods on trait based models are already present in the literature (Ippolito et al, 2012).

The development, testing and validation of (Q)SAR models requires a number of considerations in regard to quality of the data, the number and type of compounds, and the algorithms used in building and testing a model. Equally, the use of the model and interpretation of the results requires expert knowledge. More importantly, a pragmatic in silico scheme comprises the use of a combination of different methods (e.g. (Q)SAR models, Expert systems, read-across) and aims to obtain a 'weight of evidence' for a reliable and conclusive assessment.

Ecological modelling

Ecological models are the only way to fully take into account "ecology" in risk assessment. They represent an unique tool for predicting ecologically relevant effects and for extrapolating results obtained in specific sites to other sites of comparable typology. Ecological modelling practice is rapidly developing, however, their application for assessing effects of stress factors is still relatively poor, even if their relevance is strongly recognised and supported in the literature.

A strong research effort is essential to cover the gaps between academic practice and requirements for regulatory decisions, and between the potential of ecological models and population/community-level protection goals. To make full use of the potential of ecological models, a concerted action is needed to agree on standard scenarios, ecologically relevant test species and endpoints, acceptance criteria of ecological models, and to develop well-tested, flexible models that are both routinely used and improved

6.4.3. Mode-of-action studies

A much greater dependence on understanding modes of action is the central feature of the future paradigm. Such studies are research intensive. A rational framework needs to be identified to optimise progress in this vital area. Such studies will of course draw on the improved data bases and advances in in silico methodology.

6.4.4. Exposure measurement and modelling

There is a need to explore the spatial and temporal aspects of chemical fate in order to collect a more realistic picture of exposure. This can be realized collecting monitoring data at short temporal resolution (e.g. hours) on a variety of exposure scenarios. Bioavailability issues should be further investigated, especially for polar organics.

Predictive approaches should be improved developing integrated models capable to predict time and space variable concentrations in the environment (as well as for consumer and workplace exposure) to reflect the ecological and human variability of conditions and more realistically predict ecosystem and human health exposure.

7. REFERENCES

Adams RG, Lohmann R, Fernandez LA, Macfarlane JK, Gschwend PM. 2007. Polyethylene devices: Passive samplers for measuring dissolved hydrophobic organic compounds in aquatic environments. Environ Sci Technol 41: 1317-1323

Adler, S., Basketter, D., Creton, S., Pelkonen, O., van Benthem, J., Zuang, V., Andersen, K.E., Angers-Loustau, A., Aptula, A., Bal-Price, A., Benfenati, E., Bernauer, U., Bessems, J., Bois, F.Y., Boobis, A., Brandon, E., Bremer, S., Broschard, T., Casati, S., Coecke, S., Corvi, R., Cronin, M., Daston, G., Dekant, W., Felter, S., Grignard, E., Gundert-Remy, U., Heinonen, T., Kimber, I., Kleinjans, J., Komulainen, H., Kreiling, R., Kreysa, J., Leite, S.B., Loizou, G., Maxwell, G., Mazzatorta, P., Munn,S., Pfuhler, S., Phrakonkham, P., Piersma, A., Poth, A., Prieto, P., Repetto, G., Rogiers, V., Schoeters, G., Schwarz, M., Serafimova, R., Tahti, H., Testai, E., van Delft, J., van Loveren, H., Vinken, M., Worth, A., Zaldivar, J.M., 2011. Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch. Toxicol. 85, 367–485.

Allen, H. E.; Hansen, D. J. 1996. The importance of trace metal speciation to water quality criteria.Water Environ. Res. 68, 42-54.

Alvarez DA, Petty JD, Huckins JN, Jones-Lepp TL, Getting DT, Goddard JP, et al. 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ Toxicol Chem 23: 1640-1648

Armitage JM, Cousins IT, Hauck M, Harbers JB, Huijbregts MAJ. 2007. Empirical evaluation of spatial and non-spatial European-scale multimedia fate models: results and implications for chemical risk assessment, J Environ Monit 9:572–581.

Armitage JM, Gobas FAPC, 2007. A Terrestrial Food-Chain Bioaccumulation Model for POPs. . Sci. Technol., 41:4019–4025

Ashauer R, Boxall ABA, Brown CD (2006). Predicting effects on aquatic organisms from fluctuating or pulsed exposure to pesticides. Environ Toxicol Chem 25(7): 1899-1912.

Ashauer R, Boxall ABA, Brown CD (2007). Modelling combined effects of pulsed exposure to carbaryl and chlorpyrifos on Gammarus pulex. Environ Sci Technol 41:5535-5541.

Baird DJ, Baker CJO, Brua R, Hajibabaei M, McNicol K, Pascoe TJ, de Zwart D. 2011. Toward a knowledge infrastructure for traits-based ecological risk assessment. Integr Environ Assess Manag 7:209-215

Baird DJ, Van den Brink PJ. 2007. Using biological traits to predict species sensitivity to toxic substances? Ecotoxicol Environ Saf 67: 296-301.

Bednarska AJ, Portka I, Kramarz PE, Laskowski R. 2009. Combined effect of environmental pollutants (nickel, chlorpyrifos) and temperature on the ground beetle, Pterostichus oblongopunctatus (Coleoptera: Carabidae). Environ. Toxicol. Chem. 28: 864–872.

Beketov M, Schäfer R, Marwitz A, Paschke, A, Liess M. 2008. Long-term stream invertebrate community alterations induced by the insecticide thiacloprid: effect concentrations and recovery dynamics. Science of the Total Environment. 405, 96-108.

Beketov MA, Liess M (2006) The influence of predation on the chronic response of Artemia sp. populations to a toxicant. Journal of Applied Ecology 43 (6):1069-1074

Berg N, De Wever B, Fuchs HW, Gaca M, Krul C and Roggen EL (2011) Toxicology in the 21st century--working our way towards a visionary reality. Toxicology in vitro : an international journal published in association with BIBRA 25:874-881.

Bhattacharya S, Zhang Q, Carmichael PL, Boekelheide K and Andersen ME (2011) Toxicity testing in the 21 century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. PLoS One 6:e20887.

Blaauboer, B.J. (2010) Biokinetic modelling and in vitro-in vivo extrapolations. J Toxicol Environ Health B 13, 242-252.

Bois FY, Jamei M, Clewell HJ, PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. (2010) Toxicology, 278:256-67

Boobis AR (2010) Mode of action considerations in the quantitative assessment of tumour responses in the liver. Basic & clinical pharmacology & toxicology 106:173-179.

Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C (2008) IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Crit Rev Toxicol 38:87-96.

Booij K, Smedes F, van Weerlee EM. 2002. Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. Chemosphere 46: 1157-1161

Boogaard, PJ, Aylward LL, Hays SM (2012). Application of human biomonitoring (HBM) of chemical exposure in the characterisation of health risks under REACH. International journal of hygiene and environmental health 215, 238-241.

Boxall ABA, Fogg LA, Ashauer R, Bowles T, Sinclair CJ, Colyer A (2005). Assessing the ecotoxicological impact to aquatic organisms from pulsed exposures to pesticides. Cranfield University research report for Defra project PN0946, Defra, UK.

Boxall, ABA.; Brown, C. D.; Barrett, K. L. (2002). Higher-tier laboratory methods for assessing the aquatic toxicity of pesticides. Pest Manage. Sci. 2002, 58, 637-648.

Boyle, T.P., Fairchild, J.F., RobinsonWilson, E.F., Haverland, P.S., Lebo, J.A., 1996. Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. Environ. Toxicol. Chem. 15, 1806-1814.

Brock T, Alix A, Brown C, Capri E, Gottesbueren B, Heimbach F, Lythgo C, Schulz R, Streloke M 2009. Linking aquatic exposure and effects in the registration procedure of plant protection products. Society of Environmental Toxicology and Chemistry.

Brock TC, Arts G.H., Maltby L, Van den Brink PJ (2006) Aquatic risks of pesticides, ecological protection goals, and common aims in European Union legislation. Integrated Environmental Assessment and Management. 2 (4), e20-e46

Bronner G, Goss KU. 2011. Predicting Sorption of Pesticides and Other Multifunctional Organic Chemicals to Soil Organic Carbon. Environ Sci Technol 45: 1313-1319

Brownawell BJ, Chen H, Collier JM, Westall JC. 1990. Adsorption of organic cations to natural materials. Environ Sci Technol 24: 1234 - 1241

Bryant V, Newbery DM, McLusky DS, Campbell R. 1985. Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (Corophium volutator, Macoma balthica). Marine Ecology – Progress Series 24: 139-153.

Burgert S, Schäfer R, Foit K, Kattwinkel M, Metzeling M, MacEwand R, Kefford BJ, Liess M. 2011. Modelling Aquatic Exposure and Effects of Insecticides - Application to South-Eastern Australia. Science of the Total Environment. 409. 2807-2814

Calow P. (1998) Environmental Risk Assessment and Management : the Whats, Whis and Hows? In: Calow P. (Ed.) Handbook of Environmental Risk Assessment and Management. Blackwell, Oxford, UK

Carman, K.R., Bianchi, T.S., Kloep, F., 2000. Influence of grazing and nitrogen on benthic algal blooms in diesel fuel-contaminated saltmarsh sediments. Environ. Sci. Technol. 34, 107-111.

Carmichael N, Bausen M, Boobis AR, Cohen SM, Embry M, Fruijtier-Polloth C, Greim H, Lewis R, Bette Meek ME, Mellor H, Vickers C and Doe J (2011) Using mode of action information to improve regulatory decision-making: an ECETOC/ILSI RF/HESI workshop overview. Crit Rev Toxicol 41:175-186.

Carrasquillo AJ, Bruland GL, Mackay AA, Vasudevan D. 2008. Sorption of Ciprofloxacin and Oxytetracycline Zwitterions to Soils and Soil Minerals: Influence of Compound Structure. Environ Sci Technol 42: 7634-7642

Caswell H. 2001. Matrix Population Models. Sinauer, Sunderland, Massachusetts.

Chaudhry, Q., Chrétien, J., Craciun, M., Guo, G., Lemke, F., Müller, J-A, Neagu, N. Piclin, N., Pintore, M., Trundle, P. (2007) Chapter 4: Algorithms For (Q)SAR Model Building; in Quantitative Structure-Activity Relationship (QSAR) for Pesticide Regulatory Purposes, Benfenati, E. (Ed), pp 111, Elsevier. ISBN 13: 978-0444-52710-3.

Cheeseman MA, Machuga EJ and Bailey AB (1999) A tiered approach to threshold of regulation. Food Chem Toxicol 37:387-412.

Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RW and Kavlock RJ (2007) The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicol Sci 95:5-12.

Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RClewell, R.A., Clewell, H.J., 2008. Development and specification of physiologically based pharmacokinetic models for use in risk assessment. Regul. Toxicol. Pharmacol. 50, 129–143.

Coecke S., Pelkonen O. Batista Leite S., Bernauer U, Bessems JGM , Bois FY., Gundert-Remy U, Loizou G, Testai E, Zaldívar J-M Toxicokinetics as a key to the integrated toxicity risk assessment based primarily on non-animal approaches. Toxicol. in Vitro (2012), http://dx.doi.org/10.1016/j.tiv.2012.06.012

Cornelissen G, Kukulska Z, Kalaitzidis S, Christanis K, Gustafsson O. 2004. Relations between environmental black carbon sorption and geochemical sorbent characteristics. Environ Sci Technol 38: 3632-3640

Cornelissen G, Rigterink H, Hulscher EMt, Vrind BA, Noort PCMv. 2001. A simple tenax extraction method to determine the availability of sediment-sorbed organic compounds. Environ Toxicol Chem 20: 706-711

Cotterill, J.V., Chaudhry, M.Q., Matthews, W., Watkins, R. (2008) In silico assessment of toxicity of heat-generated food contaminants, Food and Chemical Toxicology 46: 1905 –1918.

Culp JM, Hose GC, Armanini DG, Dunbar MJ, Orlofske JM, Poff NL, Pollard AI, Yates AG. 2011. Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. Integrated Environmental Assessment and Management, 7, 187–197.

Danish EPA 2008. Risk assessment report on nickel andd nickel compounds. Office for Official Publications of the European Communities, Luxembourg.

De Laender F, Van Oevelen D, Middelburg, JJ , Soetaert, K . 2010a. Uncertainties in ecological, chemical and physiological parameters of a bioaccumulation model: Implications for internal concentrations and tissue based risk quotients. EES 73, 3, 240-246)

De Laender, F., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R., 2008. Validation of an ecosystem modelling approach as a tool for ecological effect assessments. Chemosphere 71, 529-545.

De Laender, F., Soetaert, K., De Schamphelaere, K.A.C., Middelburg, J.J., Janssen, C.R., 2010a. Ecological significance of hazardous concentrations in a planktonic food web. Ecotox. Environ. Safe. 73, 247-253.

De Laender, F., Soetaert, K., Middelburg, J.J., 2010b. Inferring chemical effects on carbon flows in aquatic food webs: Methodology and case study. Environ. Pollut. 158, 1775-1782.

De Laender, F., Van den Brink, P., Janssen, C., 2011. Functional redundancy and food web functioning in linuron-exposed ecosystems. Environ. Pollut. in press.

De Laender, F.; Van Oevelen, D.; Middelburg, J. J.; Soetaert, K. 2009. Incorporating ecological data and associated uncertainty in bioaccumulation modeling: methodology development and case study. Environ. Sci. Technol., 43 (7), 2620–2626

De Schamphelaere K.A.C. and Janssen C.R., 2004. Development and field validation of a biotic ligand model predicting chronic copper toxicity to Daphnia magna. Environmental Toxicology and Chemistry, 23(6), 1365-1375.

De Schamphelaere KAC, Canli M., Van Lierde V., Forrez I, Vanhaecke F, Janssen C.R. 2004. Reproductive toxicity of dietary zinc to Daphnia magna. Aquatic Toxicology. 70 (3), 233-244

Deblonde T, Cossu-Leguille C, Hartemann P., Emerging pollutants in wastewater: A review of the literature, Int J Hyg Environ Health. 2011

Debruyn AMH, Gobas FAP. 2007. The sorptive capacity of animal protein. Environ Toxicol Chem 26: 1803-1808

Demon A, Eijsackers H. 1985. The effects of lindane and azinphosmethyl on survival time of soil animals, under extreme or fluctuating temperature and moisture conditions. Z. Angew. Entomol. 100: 504-510.

Di Toro D.M., Allen H.E., Bergman H.L., Meyer J.S., Paquin P.R., Santore R.C. (2001). A biotic ligand model of the acute toxicity of metals I. Technical basis. Environ.Toxicol.Chem. 20, 2383-2396.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, et al. 1991. Technical basis for establishing sediment quality criteria for nonionic organic-chemicals using equilibrium partitioning. Environ Toxicol Chem 10: 1541-1583

Dix, D.J., Gallagher, K., Benson, W.H., Groskinsky, B.L., McClintock, J.T., Dearfield, K.L., & Farland, W.H. (2006) A framework for the use of genomics data at the EPA. Nature Biotechnology 24: 1108-1111.

Droge STJ, Yarza-Irusta L, Hermens JLM. 2009. Modeling Nonlinear Sorption of Alcohol Ethoxylates to Sediment: The Influence of Molecular Structure and Sediment Properties. Environ Sci Technol 43: 5712-5718

EC 1976, Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. OJ L262, 27.9.1976, p. 169

EC 1991. Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Annex VI – Uniform Principles for Evaluation and Authorization of Plant Protection Products. Off J Eur Comm. L 230, 19/8/1991

EC 1993, Directive 93/67/EEC of 2 July 1993 laying down the principles for the assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/548/EEC. OJ L227. 8.9.1993, p. 9

EC 1999. CSTEE Opinion on human and wildlife health effects of endocrine disrupting chemicals, with emphasis on wildlife and ecotoxicology test methods. Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), DG SANCO, Bruxelles, March 1999.

EC 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy Off. J. Eur. Comm. L 327/1 22/10/2000.

EC 2003. Technical Guidance Document (TGD) on Risk Assessment of Chemical Substances (2nd edition). European Commission, European Chemical Bureau, Joint Research Centre, EUR 20418 EN/2.

EC 2004 European Union System for the Evaluation of Substances 2.0 (EUSES 2.0). Prepared for the European Chemicals Bureau by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands (RIVM Report no. 601900005). Available via the European Chemicals Bureau, http://ecb.jrc.it,

EC 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for Community action in the field of marine environmental policy (Marine Strategy Framework Directive).Off. J. Eur. Comm. L 164/19, 25/06/2008.ECHA 2008. Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. ECHA, Helsinki, Finland.

ECHA 2008. Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals. ECHA, Helsinki, Finland.

ECI (European Copper Institute) 2006. Voluntary risk assessment report on copper, copper(II)sulphate pentahydrate, copper(I)oxide, copper(II)oxide, dicopper chloride trihydroxide. Office for Official Publications of the European Communities, Luxembourg.

EFSA Scientific Committee; Guidance on default assumptions used by the EFSA Scientific Panels and Committee, and EFSA Units in the absence of actual measured data. Available online: www.efsa.europa.eu (2011)

Eickmann U. , Eickmann J., Tischer M. (2007) Exposure to sprays – Comparison of the available exposure models, Gefahrstoffe - Reinhaltung der Luft ,67 , 105-318

Eisinger, D. & Thulke, H.-H. (2008) Spatial pattern formation facilitates eradication of infectious diseases. Journal of Applied Ecology 45: 415–423

Endo S, Escher BI, Goss KU. 2011. Capacities of Membrane Lipids to Accumulate Neutral Organic Chemicals. Environ Sci Technol 45: 5912-5921

EPER (2009) http://eper.ec.europa.eu/eper/

E-PRTR (2011) http://prtr.ec.europa.eu/

Escher BI, Ashauer R, Dyer S, Hermens JLM, Lee J-H, Leslie HA, et al. 2011a. Crucial role of mechanisms and modes of toxic action for understanding tissue residue toxicity and internal effect concentrations of organic chemicals. Integrated Environmental Assessment and Management 7: 28-49

Escher BI, Cowan-Ellsberry CE, Dyer S, Embry MR, Erhardt S, Halder M, et al. 2011b. Protein and Lipid Binding Parameters in Rainbow Trout (Oncorhynchus mykiss) Blood and Liver Fractions to Extrapolate from an in Vitro Metabolic Degradation Assay to in Vivo Bioaccumulation Potential of Hydrophobic Organic Chemicals. Chem Res Toxicol 24: 1134-1143

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

Fioroni P., Oehlmann J., Stroben E., 1991. The pseudohermaphroditism of prosobranchs; morphological aspects: Zool. Anz., 226: 1-26.

Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic ecosystems. Sci. Total Environ. 317, 207-233.

FOCUS (2001a) FOCUS groundwater scenarios in the EU review of active substances" - The report of the work of the Groundwater Scenarios Workgroup of FOCUS (FOrum

for the Co-ordination of pesticide fate models and their USe), Version 1 of November 2000. EC Document Reference Sanco/321/2000 rev.2, 202pp.

FOCUS (2001b). "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp

FOCUS 2000. FOCUS groundwater scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp

FOOTPRINT (2011) The FOOTPRINT Pesticide Properties Database, www.eu-footprint.org/ppdb.html

Forbes VE, Calow P, Grimm V, Hayashi T, Jager T, Katholm A, Palmqvist A, Pastorok R, Salvito D, Sibly RM, Spromberg J, Stark J, Stillman RA. 2011. Adding value to ecological risk assessment with population modeling. Human and Ecological Risk Assessment 17: 287-299

Forbes VE, Calow P, Grimm V, Hayashi T, Jager T, Palmqvist A, Pastorok R, Salvito D, Sibly R, Spromberg J, Stark J, Stillman R. 2010. Integrating population modeling into ecological risk assessment. Integrated Environmental Assessment and Management 6: 191-193

Forbes VE, Hommen U, Thorbek P, Heimbach F, Van den Brink P, Wogram J, Thulke HH, Grimm V. 2009. Ecological models in support of regulatory risk assessments of pesticides: developing a strategy for the future. Integrated Environmental Assessment and Management 5:167-172

Franco A, Ferranti A, Davidsen C, Trapp S. 2010. An unexpected challenge: ionizable compounds in the REACH chemical space. International Journal of Life Cycle Assessment 15: 321-325

Friberg-Jensen, U., Wendt-Rasch, L., Woin, P., Christoffersen, K., 2003. Effects of the pyrethroid insecticide, cypermethrin, on a freshwater community studied under field conditions. I. Direct and indirect effects on abundance measures of organisms at different trophic levels. Aquat. Toxicol. 63, 357-371.

Galic, N., Hommen, U., Baveco, J.M., van den Brink, P.J. (2010) Potential application of population models in the European ecological risk assessment of chemicals II: Review of models and their potential to address environmental protection aims. Integrated Environmental Assessment and Management 6: 338–360

Gasic, B.; Moeckel, C.; MacLeod, M.; Brunner, J.; Scheringer, M.; Jones, K.C.; Hungerbühler, K. 2009. Measuring and modeling short-term variability of PCBs in air and characterization of urban source strength in Zurich, Switzerland. Environ. Sci. Technol. 43, 769-776.

Gobas FAPC (2000) Bioconcentration and Biomagnification in the Aquatic environment, In: Boethling R.S.., Mackay D. (Eds), Handbook of Property Estimation Methods, Lewis Pubs., Boca Raton, p. 481

Gobas FAPC, Zhang X, Wells. R. 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ Sci Technol 27: 2855-2863

Goss K-U, Schwarzenbach RP. 2001. Linear free energy relationships used to evaluate equilibrium partittioning of organic compounds. Environ Sci Technol 35: 1-9

Griffin JL (2003) Metabonomics: NMR spectroscopy and pattern recognition analysis of body fluids and tissues for characterisation of xenobiotic toxicity and disease diagnosis. Current opinion in chemical biology 7:648-654.

Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, Goss-Custard J, Grand T, Heinz SK, Huse G, Huth A, Jepsen JU, Jørgensen C, Mooij WM, Müller B, Pe'er G,

Piou C, Railsback SF, Robbins AM, Robbins MM, Rossmanith E, Rüger N, Strand E, Souissi S, Stillman RA, Vabø R, Visser U, DeAngelis DL. 2006. A standard protocol for describing individual-based and agent-based models. Ecological Modelling 198:115-126

Grimm V, Berger U, DeAngelis DL, Polhill G, Giske J, Railsback SF. 2010. The ODD protocol: a review and first update. Ecological Modelling 221: 2760-2768

Grimm V, Dorndorf N, Frey-Roos F, Wissel C, Wyszomirski T, Arnold W (2003) Modelling the role of social behavior in the persistence of the alpine marmot Marmota marmota. Oikos 102:124-136

Grimm V, Railsback SF. 2005. Individual-based Modeling and Ecology. Princeton University Press, Princeton, N.J., 428 pp.

Grimm V, Railsback SF. 2012. Pattern-oriented modelling: a "multiscope" for predictive systems ecology. Philosophical Transactions Royal Society London B 367: 298-310

Grimm V, Revilla E, Berger U, Jeltsch F, Mooij WM, Railsback SF, Thulke H-H, Weiner J, Wiegand T, DeAngelis DL (2005) Pattern-oriented modeling of agent-based complex systems: lessons from ecology. Science 310: 987-991

Grimm V. 2010. Short introduction to ecological modeling. In: Thorbek P, Forbes V, Heimbach F, Hommen U, Thulke HH, van den Brink PJ, Wogram J, Grimm V (eds). Ecological models for regulatory risk assessments of pesticides: developing a strategy for the future. Pensacola and Boca Raton (FL): Society of Environmental Toxicology and Chemistry (SETAC) and CRC Press, pp. 15-26.

Gülden M, Morchel S, Tahan S, Seibert H. 2002. Impact of protein binding on the availability and cytotoxic potency of organochlorine pesticides and chlorophenols in vitro. Toxicology 175: 201-213

Gülden M, Seibert H, Voss JU. 1994. Inclusion of physicochemical data in quantitative comparisons of in vitro and in vivo toxic potencies. Atla-Alternatives to Laboratory Animals 22: 185-192

Gülden, M., Morchel, S., Seibert, H., 2001. Factors influencing nominal effective concentrations of chemical compounds in vitro: cell concentration. Toxicol. In Vitro 15, 233–243.

Gustafsson O, Haghseta F, Chan C, MacFarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. Environ Sci Technol 31: 203-209

Hanazato, T., 1998. Response of a zooplankton community to insecticide application in experimental ponds: a review and the implications of the effects of chemicals on the structure and functioning of freshwater communities. Environ. Pollut. 101, 361-373.

Hansen KM, Christensen JH, Brandt J, Frohn LM, Geels C. 2004. Modelling atmospheric transport of a-hexachlorocyclohexane in the Nothern Hemisphere with a 3-D dynamic model: DEHM-POP. Atmospheric Chemistry and Physics, 4 (4), 1125-1137

Hawthorne SB, St Germain RW, Azzolina NA. 2008. Laser-induced Fluorescence Coupled with Solid-Phase Microextraction for In Situ Determination of PAHs in Sediment Pore Water. Environ Sci Technol 42: 8021-8026

Hayward, S.J., Gouin, T., Wania, F. Comparison of Four Active and passive Sampling Techniques for Pesticides in Air, Environ. Sci. Technol. 2010, 44, 3410–3416.

Heijerick DG, De Schamphelaere KAC and CR Janssen, (2002). Predicting acute zinc toxicity for Daphnia magna as a function of key water chemistry characteristics: Development and validation of a Biotic Ligand Model. Environmental Toxicology and Chemistry 21, 1309-1315.

Hendriks AJ, Traas TP, Huijbregts MAJ. 2005. Critical body residues linked to octanol-water partitioning, organism composition, and LC50 QSARs: Meta-analysis and model. Environ Sci Technol 39: 3226-3236

Hendriks, A. J.; van der Linde, A.; Cornelissen, G.; Sijm, D. 2001. The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight. Environ. Toxicol. Chem. 20 (7), 1399–1420

Heringa MB, Pastor D, Algra J, Vaes WHJ, Hermens JLM. 2002. Negligible depletion Solid-Phase Microextraction with radiolabeled analytes to study free concentrations and protein binding: An example with [3H]Estradiol. Anal Chem 74: 5993-5997

Heringa MB, Schreurs RHMM, Busser F, van der Saag P, van der Burg B, Hermens JLM. 2004. Toward more useful in vitro toxicity data with measured free concentrations. Environ Sci Technol 38: 6263-6270

Hermens JLM, Heringa MB, ter Laak TL. 2007. Bioavailability in dose and exposure assessment of organic contaminants in (Eco)toxicology. Journal of Toxicology and Environmental Health-Part a-Current Issues 70: 727-730

Heugens EHW, Hendriks AJ, Dekker T, Van Straalen NM, Admiraal W. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical Reviews in Toxicology 31 (3) , 247-284

Higgins CP, Luty RG. 2007. Modeling sorption of anionic surfactants onto sediment materials: An a priori approach for perfluoroalkyl surfactants and linear alkylbenzene sulfonates. Environ Sci Technol 41: 3254-3261

Holmstrup M, Petersen BF, Larsen MM. 1998. Combined effects of copper, desiccation, and frost on the viability of earthworm cocoons. Envrion. Tox. Chem. 17: 897-901.

Hommen U., Baveco JM, Galic,JN., van den Brink PJ 2010. Potential Application of Ecological Models in the European Environmental Risk Assessment of Chemicals I: Review of Protection Goals in EU Directives and Regulations. Integrated Environmental Assessment and Management, 6, 325–337

Horrigan, N. and D.J. Baird. 2008. Trait patterns of aquatic insects across gradients of flow-related factors. Canadian Journal of Fisheries and Aquatic Sciences. 65(4):670-680.

Hou T, Li Y, Zhang W, Wang J. Comb Chem High Throughput Screen 2009; 12(5):497-506,

Huckins JN, Manuweera GK, Petty JD, Mackay D, Lebo JA, 1993. Lipid-containing semipermeable membrane devices for monitoring organic contaminants in water. Environmental Science and Technology, 27, 2489-2496

Huckins JN, Tubergen MW, Manuweera GK. 1990. Semipermeable membrane devices containing model lipids - a new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioaccumulation potential. Chemosphere 20: 533-552

Huet M., Paulet Y. M., Glémarec M. 1996. Tributhyltin pollution in the coastal waters of west Brittany as indicated by imposex in Nucella lapillus. Mar. Environ. Res., 41: 157-167.

Huth, A., Drechsler, M., Köhler, P. (2005) Evaluation of reduced impact logging using multicriteria decision analysis. Forest Ecology and Management 207, 215-213

IPCS (International Programme on Chemical Safety), World Health Organization. Biomarkers and risk assessment: validity and validation. Geneva: World Health Organization; 2001. (Environmental Health Criteria, 222).

IPCS, 2004. IPCS risk assessment terminology. Part 2: IPCS glossary of key exposure assessment terminology. Geneva, World Health Organization, International Programme on Chemical Safety (IPCS Harmonization Project Document No. 1; http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf).

IPCS, 2011. Identifying Important Life Stages for Monitoring and Assessing Risks from Exposures to Environmental Contaminants - Consultation Draft. Geneva, World Health Organization, International Programme on Chemical Safety (IPCS) . Prepared by WHO Planning Group Members: Elaine A Cohen Hubal (Chair); Thea de Wet; Lilo Du Toit; Michael P Firestone; Mathuros Ruchirawat; Jacqueline van Engelen; Ruth A Etzel; Carolyn Vickers (WHO Secretariat).

Ippolito A, Sala S, Faber JH, Vighi M 2010. Ecological vulnerability analysis: A river basin case study. Science of the Total Environment 408: 3880-3890

Ippolito A., Todeschini R., Vighi M. (2012) Sensitivity assessment of freshwater macroinvertebrates to pesticides using biological traits. Ecotoxicology 21:336–352

Jager T, Fleuren R, Hogendoorn EA, De Korte G. 2003. Elucidating the routes of exposure for organic chemicals in the earthworm, Eisenia andrei (Oligochaeta). Environ Sci Technol 37: 3399-3404

Jager T, Kooijman SALM (2005): Modelling receptor kinetics in the analysis of survival data for organophosphorus pesticides. Environ Sci Technol 39:8307-8314.

Jager, T.; Albert, C.; Preuss, T. G.; Ashauer, R. (2011) General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology. Environmental Science & Technology 45: 2529-2540.

Jonker MTO, Koelmans AA. 2001. Polyoxymethylene solid phase extraction as a partitioning method for hydrophobic organic chemicals in sediment and soot. Environ Sci Technol 35: 3742-3748

Jonker MTO, Koelmans AA. 2002. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in the aqueous environment mechanistic considerations. Environ Sci Technol 36: 3725-3734

Jonker MTO, van der Heijden SA, Kreitinger JP, Hawthorne SB. 2007. Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with solid-phase microextraction. Environ Sci Technol 41: 7472-7478

Jonker MTO, van der Heijden SA. 2007. Bioconcentration factor hydrophobicity cutoff: An artificial phenomenon reconstructed. Environ Sci Technol 41: 7363-7369

Julien E, Boobis AR and Olin SS (2009) The Key Events Dose-Response Framework: a cross-disciplinary mode-of-action based approach to examining dose-response and thresholds. Critical reviews in food science and nutrition 49:682-689.

Kallander DB, Fisher SW, Lydy MJ (1997) Recovery following pulsed exposure to organophosphorus and carbamate insecticides in the midge, Chironomus riparius. Arch Environ Contam Toxicol 33, 29-33.

Karickhoff SW, Brown DS, Scott TA. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res 13: 241-248

Katayama A., Bhula R., Burns G.R., Carazo E., Felsot A., Hamilton D., Harris C., Kim Y.H., Kleter G., Koedel W., Linders J., Peijnenburg J G M.W., Sabljic A., Stephenson R.G., Racke D. K., Rubin B, Tanaka K., Unsworth J., Wauchope R.D. (2010) Bioavailability of Xenobiotics in the Soil Environment, Reviews of Environmental Contamination and Toxicology 203, 1-86.

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, Knudsen T, Martin M, Padilla S, Reif D, Richard A, Rotroff D, Sipes N and Dix D (2012) Update on

EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. Chemical research in toxicology 25:1287-1302.

Keller DA, Juberg DR, Catlin N, Farland WH, Hess FG, Wolf DC and Doerrer NG (2012) Identification and characterization of adverse effects in 21st century toxicology. Toxicol Sci 126:291-297.

Kelly BC, Gobas FAPC. 2003 An Arctic Terrestrial Food-Chain Bioaccumulation Model for Persistent Organic Pollutants, Environ. Sci. Technol., 37: 2966–2974

Kersting, K., Van Wijngaarden, R.P.A., 1999. Effects of a pulsed treatment with the herbicide afalon (active ingredient linuron) on macrophyte-dominated mesocosms. I. Responses of ecosystem metabolism. Environ. Toxicol. Chem. 18, 2859-2865.

Kia R, Sison RL, Heslop J, Kitteringham NR, Hanley N, Mills JS, Park BK, Goldring CE, Stem cell-derived hepatocytes as a predictive model for drug-induced liver injury: are we there yet? Br J Clin Pharmacol; 2012 doi: 10.1111/j.1365-2125.2012.04360.x.

Kipka U, Di Toro DM. 2009. Technical Basis for Polar and Nonpolar Narcotic Chemicals and Polycyclic Aromatic Hydrocarbon Criteria. III. A Polyparameter Model for Target Lipid Partitioning. Environ Toxicol Chem 28: 1429-1438

Kleinstreuer NC, Smith AM, West PR, Conard KR, Fontaine BR, Weir-Hauptman AM, Palmer JA, Knudsen TB, Dix DJ, Donley EL and Cezar GG (2011) Identifying developmental toxicity pathways for a subset of ToxCast chemicals using human embryonic stem cells and metabolomics. Toxicol Appl Pharmacol 257:111-121.

Knillmann S, Stampfli NC, Noskov YA, Beketov MA. Liess M, 2012. Interspecific competition delays recovery of Daphnia spp. populations from pesticide stress. Ecotoxicology, 21, 1039-1049

Knudsen, L.E.; Loft, S.H. and Autrup, H.: Risk assessment: the importance of genetic polymorphisms in man. Mutat. Res. 482 (2001), pp.83–88.

Kolkwitz R, Marsson M. 1902. Grundsätze für die biologische Beurteilung des Wassers nach seiner Flora und Fauna. Mitteilungen der königlichen Prüfanstalt Wasserversorgung Abwasserbeseitigung Berlin-Dahlem 1: 33-72.

Kooijman, S. A. L. M. 2010. Dynamic Energy Budget theory for metabolic organisation. Cambridge University Press.

Koshikawa, H., Xu, K.Q., Liu, Z.L., Kohata, K., Kawachi, M., Maki, H., Zhu, M.Y., Watanabe, M., 2007. Effect of the water-soluble fraction of diesel oil on bacterial and primary production and the trophic transfer to mesozooplankton through a microbial food web in Yangtze estuary, China. Estuar. Coast. Shelf Sci. 71, 68-80.

Kramer NI, Busser FJM, Oosterwijk MTT, Schirmer K, Escher BI, Hermens JLM. 2010. Development of a partition-controlled dosing system for cell assays. Chem Res Toxicol 23: 1806-1814

Kramer NI, Hermens JLM, Schirmer K. 2009. The influence of modes of action and physicochemical properties of chemicals on the correlation between in vitro and acute fish toxicity data. Toxicol in Vitro 23: 1372-1379

Krewski D, Andersen ME, Mantus E and Zeise L (2009) Toxicity testing in the 21st century: implications for human health risk assessment. Risk Anal 29:474-479.

Kwon JH, Wuethrich T, Mayer P, Escher BI. 2009. Development of a dynamic delivery method for in vitro bioassays. Chemosphere 76: 83-90

Landrum PF, Lotufob GR, Gossiauxa DC, Gedeonc ML, Leed J-H. 2003. Bioaccumulation and critical body residue of PAHs in the amphipod, Diporeia spp.: additional evidence to support toxicity additivity for PAH mixtures. Chemosphere 51: 481-489

Lee, R.G.M., Hung, H., Mackay, D., Jones, K.C., 1998. Measurement and modeling of the diurnal cycling of atmospheric PCBs and PAHs. Environ. Sci. Technol. 32: 2172-2179

Liebig, M., Schmidt, G., Bontje, D., Kooi, B.W., Streck, G., Traunspurger, W., Knacker, T., 2008. Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor microcosm. Aquat. Toxicol. 88, 102-110.

Liess M, Beketov M. 2011. Traits and stress - keys to identify community effects of low levels of toxicants in test systems. Ecotoxicology. Online First. Ecotoxicology. 20(6). 1328-1340.

Liess M, Brown C, Dohmen P, Duquesne S, Heimbach F, Kreuger J, Lagadic L, Reinert W, Maund S, Streloke M, Tarazona J (2005) Effects of Pesticides in the Field – EPIF. SETAC Press, Brussels, Belgium (http://www.systemecology.eu/EPiF/Download_files/EPiF.pdf)

Liess M, Champeau O, Riddle M, Schulz R, Duquesne S (2001) Combined effects of ultraviolett-B radiation and food shortage on the sensitivity of the Antarctic amphipod Paramoera walkeri to copper. Environmental Toxicology and Chemistry 20 (9):2088-2092

Liess M, Foit K. 2010. Intraspecific competition delays recovery of population structure. Aquatic Toxicology. 97, 15-22.

Liess M, Kattwinkel M, Kaske O, Beketov M, Steinicke H, Scholz M, Henle K. 2010. Considering protected aquatic non-target species in the environmental risk assessment of plant protection products. UBA report. http://www.ufz.de/export/data/1/24952_2010_Protected_Species_UBA.pdf

Liess M, Schäfer RB, Schriever CA (2008) The footprint of pesticide stress in communities - species traits reveal community effects of toxicants. Science of the Total Environment 406 (3):484-490

Liess M, Schulz R (1999) Linking insecticide contamination and population response in an agricultural stream. Environmental Toxicology and Chemistry 18:1948-1955

Liess M, von der Ohe PC. 2005. Analyzing effects of pesticides on invertebrate communities in streams. Environ Toxicol Chem 24(4): 954-965.

Liess, M., 2002. Population response to toxicants is altered by intraspecific interaction. Environmental Toxicology and Chemistry. 21 (1), 138-142.

Liess, M., R. Schulz, M.H.-D. Liess, B. Rother & R. Kreuzig, 1999. Determination of insecticide contamination in agricultural headwater streams. Water Research 33: 239-247.

Loizou G, Hogg A, MEGen: A Physiologically Based Pharmacokinetic Model Generator. Front Pharmacol. 2011;2:56.

Ma, J., Daggupaty, S., Harner, T., Li, Y., 2003. Impacts of lindane usage in the Canadian prairies on the Great Lakes ecosystem. 1. Coupled atmospheric transport model and modeled concentrations in air and soil. Environ. Sci. Technol. 37, 3774-3781

Macedo-Sousa JA, Soares AM, Tarazona JV. A conceptual model for assessing risks in a Mediterranean Natura 2000 Network site. Sci Total Environ. 2009 Jan 15;407(3):1224-31

Mackay AA, Seremet DE. 2008. Probe Compounds to Quantify Cation Exchange and Complexation Interactions of Ciprofloxacin with Soils. Environ Sci Technol 42: 8270-8276

Mackay D. Fraser A. 2000. Bioaccumulation of persistent organic chemicals: mechanisms and models Environ. Poll. 110:375-391

Mackay D., Di Guardo A., Paterson S., Cowan C. E.(1996a): Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ. Toxicol. Chem.,15,1627-1637.

Mackay D., Di Guardo A., Paterson S., Kicsi G., Cowan C. E. (1996b): Assessing the Fate of New and Existing Chemicals: a Five Stage Process. Environ. Toxicol. Chem.,15,1618-1626.

Mackay D., Di Guardo A., Paterson S., Kicsi G., Cowan C.E., Kane D. M. (1996c): Assessment of Chemical Fate in the Environment Using Evaluative, Regional and Local-Scale Models: Illustrative Application to Chlorobenzene and Linear Alkylbenzene Sulfonates. Environ. Toxicol. Chem.,15,1638-1648

Mackay D., Mackay N. (2007) Mathematical Models of Chemical Transport and Fate, in Suter G.W. II (Ed) Ecological Risk Assessment,, CRC Press, Boca Raton, p. 643.

Macleod SL, McClure EL, Wong CS. 2007. Laboratory calibration and field deployment of the polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water. Environ Toxicol Chem 26: 2517-2529

Malazizi, L., Neagu, D., Chaudhry, Q. (2006) A Data Quality Assessment Algorithm with Application in Predictive Toxicology, TASK Quarterly Journal (Scientific Bulletin of Academic Computer Center in Gdansk, Poland), ISSN 1428-6394, volume 11, number 1-2/2007 Advances in Artificial Intelligence, www.task.gda.pl/quart/07-1-2.html

Maltby L, Blake N, Brock TCM, Van Den Brink PJ, 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environmental Toxicology and Chemistry 24 (2) ,379-388

Mandalakis M, Berresheim H, Stephanou EG, 2003. Direct evidence for destruction of polychlorobiphenyls by OH radicals in the subtropical troposphere. Environmental Science and Technology 37 (3), 542-547

Martin OV, Lester JN, Voulvoulis N and Boobis AR (2007) Human health and endocrine disruption: a simple multicriteria framework for the qualitative assessment of end point specific risks in a context of scientific uncertainty. Toxicol Sci 98:332-347.

Mayer P, Holmstrup M. 2008. Passive dosing of soil invertebrates with polycyclic aromatic hydrocarbons: Limited chemical activity explains toxicity cutoff. Environ Sci Technol 42: 7516-7521

Mayer P, Vaes WHJ, Wijnker F, Legierse KCHM, Kraaij R, Tolls J, et al. 2000. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid phase microextraction fibers. Environ Sci Technol 34: 5177-5183

McCarty LS, Landrum PF, Luoma SN, Meador JP, Merten AA, Shephard BK, et al. 2011. Advancing environmental toxicology through chemical dosimetry: External exposures versus tissue residues. Integrated Environmental Assessment and Management 7: 7-27

McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 27: 1719-1728

McLachlan, M.S., 1996. Bioaccumulation of hydrophobic chemicals in agricultural food chains. Environ. Sci. Technol.30: 252-259.

Moeckel C., Nizzetto L., Di Guardo A., Steinnes E., Freppaz M., Filippa G., Camporini P., Benner J., Jones K.C. (2008) Persistent organic pollutants in boreal and montane soil profiles: distribution, evidence of processes and implications for global cycling,: Environ. Sci. Technol. 42, 8374–8380

Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G (2010) Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. Ecotoxicology 19 (1):207-215.

Morselli M., Ghirardello D., Semplice M., Di Guardo A. 2011 Modeling short-term variability of semivolatile organic chemicals in air at a local scale: An integrated modeling approach Environ. Poll., 159, 1406-1412.

Munro IC, Renwick AG and Danielewska-Nikiel B (2008) The Threshold of Toxicological Concern (TTC) in risk assessment. Toxicol Lett 180:151-156.

Naes K, Axelman J, Naf C, Broman-D. 1998. Role of soot carbon and other carbon matrices in the distribution of PAHs among particles, DOC, and the dissolved phase in the effluent and recipient waters of an Aluminum reduction plant. Environ Sci Technol 32: 1786-1792

Naito, W., Miyamoto, K., Nakanishi, J., Masunaga, S., Bartell, S.M., 2003. Evaluation of an ecosystem model in ecological risk assessment of chemicals. Chemosphere 53, 363-375.

Netzeva TI, Worth AP, Aldenberg T, Benigni R, Cronin MTD, Gramatica P, Jaworska JS, Kahn S, Klopman G, Marchant CA, Myatt G, Nikolova-Jeliazkova N, Patlewicz GY, Perkins R, Roberts DW, Schultz TW, Stanton DT, van de Sandt JJM, Tong W, Veith G, Yang C 2005. Current status of methods for defining the applicability domain of (Quantitative) Structure- Activity Relationships. ATLA 33: 155-173.

Nichols JW, Fitzsimmons PN, Whiteman FW, Dawson TD, Babeu L, Juenemann J. 2004. A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic compounds by fish - I. Feeding studies with 2,2 ',5,5 '-tetrachlorobiphenyl. Toxicol Sci 77: 206-218

Nichols JW, Schultz IR, Fitzsimmons PN. 2006. In vitro-in vivo extrapolation of quantitative hepatic biotransformation data for fish - I. A review of methods, and strategies for incorporating intrinsic clearance estimates into chemical kinetic models. Aquat Toxicol 78: 74-90

Nienstedt KM, Brock TCM, Wensem J, Montforts M, Hart A, Aagaard A, Alix A, Boesten J, Bopp SK, Brown C, Capri E, Forbes V, Köpp H, Liess M, Luttik R, Maltby L, Sousa JP, Streissl F, Hardy AR. 2012. Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Science of the Total Environment. 415. 31-38.

Nisbet RM, Muller EB, Lika K, Kooijman SALM. 2000. From molecules to ecosystems through dynamic energy budget models. Journal of Animal Ecology 69: 913-926

Nizzetto L., Cassani C., Di Guardo A. 2006b. Deposition of PCBs in Mountains: The Forest Filter Effect of Different Forest Ecosystem Types. Ecotoxicol. Environ. Saf., 63, pp. 75-83.

Nizzetto L., Jarvis A., Brivio P.A., Jones K.C., Di Guardo A. 2008b. Seasonality of air-forest canopy POP exchange, Environ. Sci. Technol. 42, 8778–8783.

Nizzetto L., Jones K.C., Gramatica P., Papa E., Cerabolini B., Di Guardo A. 2006a. Accumulation of Persistent Organic Pollutants (POPs) in canopies of different forest types: role of species composition and altitudinal-temperature gradient, Environ. Sci. Technol., 40, 6580-6586

Nizzetto L., Pastore C., Liu X., Camporini P., Stroppiana D., Herbert B., Boschetti M., Zhang G., Brivio P. A., Jones K. C., Di Guardo A. 2008a. Accumulation parameters and seasonal trends for PCBs in temperate and boreal forest plant species, Environ. Sci. Technol., 42, 5911–5916.

Nizzetto, L.; MacLeod, M.; Borgå, K.; Cabrerizo, A.; Dachs, J.; Di Guardo, A.; Ghirardello, D.; Hansen, K.M.; Jarvis, A.; Lindroth, A.; Ludwig, B.; Monteith, D.; Perlinger, J.A.;

Scheringer, M.; Schwendenmann, L.; Semple, K.T.; Wick, L.Y.; Zhang, G.; Jones, K.C. (2010) Past, present and future controls on levels of persistent organic pollutants in the global environment. Environ. Sci. Technol. 44, 6526–6531

Nowack B., Bucheli T.D., (2007) Occurrence, behavior and effects of nanoparticles in the environment, Environ. Poll. 150 5-22.

OECD 2000 OECD SERIES ON TESTING AND ASSESSMENT, Number 18, Report of the OECD Workshop on Improving the Use of Monitoring Data in the Exposure Assessment of Industrial Chemicals),ENV/JM/MONO(2000)2, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282000%292&docLanguage=En

OECD, 2011. OECD QSAR Toolbox. http://www.qsartoolbox.org/project.html

Oke, T.R., 1987. Boundary Layer Climates, second ed. Routledge, London, U.K.

Otto S. P., and T. Day. 2007. A Biologist's Guide to Mathematical Modeling in Ecology and Evolution. Princeton University Press, Princeton, NJ.

Park M.V.D.Z.,. Delmaar J.E and van Engelen J.G.M. (2006) Comparison of consumer exposure modelling tools, Inventory of possible improvements of ConsExpo. RIVM report 320104006/2006

Peck, S. L. 2004. Simulation as experiment: a philosophical reassessment of biological modeling. Trends in Ecology and Evolution 19:530-534. Peeters ETHM, Garderniers JJP. 1994. New methods to assess the ecological status of surface waters in The Netherlands. Part 1: Running Waters. Verh Internat Verein Limnol 25: 1914-1916.

Peeters ETHM, Garderniers JJP. 1994. New methods to assess the ecological status of surface waters in The Netherlands. Part 1: Running Waters. Verh Internat Verein Limnol 25: 1914-1916.

Petersen, D.G., Sundback, K., Larson, F., Dahllof, I., 2009. Pyrene toxicity is affected by the nutrient status of a marine sediment community: Implications for risk assessment. Aquat. Toxicol. 95, 37-43.

Pieters BJ, Paschke A, Reynaldi S, Kraak MHS, Admiraal W, Liess M 2005. Influence of food limitation on the effects of Fenvalerate pulse exposure on the life history and population growth rate of Daphnia magna. Environmental Toxicology and Chemistry 24 (9):2254-2259

Pistocchi A., Groenwold J., Lahr J., Loos M., Mujica M., Ragas A. M. J., Rallo R., Sala S., Schlink U., Strebel K., Vighi M., Vizcaino P. 2011. Mapping Cumulative Environmental Risks: Examples from the EU NoMiracle Project. Environ Model Assess., 16: 119-133

Pleil JD, Sheldon LS (2011). Adapting concepts from systems biology to develop systems exposure event networks for exposure science research. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals 16, 99-105.

Poff NL, Olden JD, Vieira NKM, Finn DS, Simmons MP, Kondratieff BC. 2006. Functional trait niches of North American lotic insects: trait-based ecological applications in light of phylogenetic relationships. J N Am Benthol Soc 25: 730-755.

Poiger H, Schlatter C (1986). Pharmacokinetics of 2,3,7,8-TCDD in man. Chemosphere 15, 9-12.

Poynton, H.C., Loguinov, A.V., Varshavsky, J.R., Chan, S., Perkins, E.J., & Vulpe, C.D. 2008. Gene expression profiling in Daphnia magna Part I: Concentration-dependent profiles provide support for the no observed transcriptional effect level. Environmental Science and Technology 42: 6250-6256.

Pretzsch, H., Biber, P. and Dursky, J. (2002) The single tree-based stand simulator SILVA: construction, application and evaluation. Forest Ecology and Management 162: 3-21

Price, N. and Chaudhry, Q. (2011) QSAR and Expert Systems as Tools in Bioremediation, Organic xenobiotics and plants - from the cellular to the ecosystem level, Schröder, P. and Collins, C.D. (Eds.), Springer Ecophysiology Series, ISBN: 978-90-481-9851-1.

Quik JTK, Martien Cohen Stuart, Marja Wouterse, Willie Peijnenburg, A. Jan Hendriks and Dik van de Meent (2011a). Natural colloids are the dominant factor in sedimentation of nanoparticles. Submitted.

Quik JTK, S Foss Hansen, A Baun, D van de Meent (2011b). How to assess exposure of aquatic organisms to manufactured nanoparticles? Environment International, in press.

Railsback S.F., Harvey B.C., Hayse J.W. & LaGory K.E. 2005. Tests of theory for diel variation in salmonid feeding activity and habitat use. Ecology, 86, 947–959.Reichenberg F, Mayer P. 2006. Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. Environ Toxicol Chem 25: 1239-1245

Railsback SF, Harvey BC (2002) Analysis of habitat-selection rules using an individual-based model. Ecology 83: 1817-1830

Reichenberg F, Mayer P. 2006. Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. Environ Toxicol Chem 25: 1239-1245

Reid BJ, Stokes JD, Jones KC, Semple KT. 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environ Sci Technol 34: 3174-3179

Reinert KH, Giddings JM, Judd L (2002). Effects analysis of timevarying or repeated exposures in aquatic ecological risk assessment of agrochemicals. Environ Toxicol Chem 21, 1977-1992.

Relyea R, Hoverman J (2006) Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecology Letters 9 (10):1157-1171

Relyea, R.A., Diecks, N., 2008. An unforeseen chain of events: Lethal effects of pesticides on frogs at sublethal concentrations. Ecol. Appl. 18, 1728-1742.

Relyea, R.A., Hoverman, J.T., 2008. Interactive effects of predators and a pesticide on aquatic communities. Oikos 117, 1647-1658.

Reynaldi S, Meiser M. Liess M. 2011. Effects of the pyrethroid fenvalerate on the alarm response and on the vulnerability of the mosquito larva Culex pipiens molestus to the predator Notonecta glauca. Aquatic Toxicology. 104(1-2). 56-60.

Richter MK, Sander M, Krauss M, Christl I, Dahinden MG, Schneider MK, et al. 2009. Cation binding of antimicrobial sulfathiazole to Leonardite humic acid. Environ Sci Technol 43: 6632-6638

Rico-Rico A, Droge STJ, Hermens JLM. 2010. Predicting Sediment Sorption Coefficients for Linear Alkylbenzenesulfonate Congeners from Polyacrylate-Water Partition Coefficients at Different Salinities. Environ Sci Technol 44: 941-947

Rotroff DM, Wetmore BA, Dix DJ, Ferguson SS, Clewell HJ, Houck KA, Lecluyse EL, Andersen ME, Judson RS, Smith CM, Sochaski MA, Kavlock RJ, Boellmann F, Martin MT, Reif DM, Wambaugh JF and Thomas RS (2010) Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicol Sci 117:348-358.

Rubach MN, Baird DJ, Van Den Brink PJ. 2010. A new method for ranking mode-specific sensitivity of freshwater arthropods to insecticides and ist relationship to biological traits. Environmental Toxicology and Chemistry 29 (2) , pp. 476-487

Rubach, M.N., Ashauer, R., Buchwalter, D.B., De Lange, H.J., Hamer, M., Preuss, T.G., Töpke, K. & Maund, S.J. 2011. A Framework for Trait-based Assessment in Ecotoxicology. Integrated Environmental Assessment and Management, 7, 172–186.

Rubach, M.N., D.J. Baird, M-C. Boerwinkel, S.J. Maund, I. Roessink and P.J. Van den Brink (2012). Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos. Ecotoxicology 21: 2088-2101.

Rykiel, E. J. 1996. Testing ecological models: the meaning of validation. Ecological Modelling 90:229-244.

Sabljic A, Güsten H, Verhaar HJM, Hermens JLM. 1995. QSAR modelling of soil sorption. Improvements and systematics of log Koc vs. log Kow correlations. Chemosphere 31: 4489-4514

Sala S., Vighi M., (2008) GIS-based procedure for site-specific risk assessment of pesticides for aquatic ecosystems. Ecotox. Environ. Saf., 69(1), 1-12.

Sander M, Pignatello JJ. 2007. On the reversibility of sorption to black carbon: Distinguishing true hysteresis from artificial hysteresis caused by dilution of a competing adsorbate. Environmental Science & Technology 41: 843-849

Schäfer R, Caquet T, Siimes K, Mueller, R, Lagadic L, Liess M. 2007. Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Science of the Total Environment. 382, 272-285.

Schauer UM, Volkel W, Dekant W (2006a). Toxicokinetics of tetrabromobisphenol a in humans and rats after oral administration. Toxicological Sciences 9**1**, 49-58.

Schauer UM, Volkel W, Heusener A, Colnot T, Broschard TH, von Landenberg F, Dekant W (2006b). Kinetics of 3-(4-methylbenzylidene)camphor in rats and humans after dermal application. Toxicology Applied Pharmacology 216, 339-346.

SCHER, SCCS, SCENIHR, 2011. Opinion on the Toxicity and Assessment of Chemical Mixtures. December 2011

SCENIHR 2009 (Scientific Committee on Emerging and Newly Identified Health Risks), Risk assessment of products of nanotechnologies, 19 January 2009

SCENIHR 2012 (Scientific Committee on Emerging and Newly Identified Health Risks), Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty,19 March, 2012 http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_s_001.pdf

Schirmer K. 2006. Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish. Toxicology 224: 163-183

Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010b. Ecological modelling supporting environmental decision making: a strategy for the future. Trends in Ecology and Evolution 25: 479-486.

Schmolke V, Thorbek P, Chapman P, Grimm V. 2010a. Ecological modelling and pesticide risk assessment: a review of current modelling practice. Environmental Toxicology and Chemistry 29: 1006-1012.

Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, et al. 2006. The challenge of micropollutants in aquatic systems. Science 313: 1072-1077

Sehili, A.M., Lammel, G., 2007. Global fate and distribution of polycyclic aromatic hydrocarbons emitted from Europe and Russia. Atmos. Environ. 41 (37), 8301-8315.

Selck, H., Riemann, B., Christoffersen, K., Forbes, V.E., Gustavson, K., Hansen, B.W., Jacobsen, J.A., Kusk, O.K., Petersen, S., 2002. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. Ecotox. Environ. Safe. 52, 97-112.

Semple KT, Doick KJ, Jones KC, Burauel P, Craven A, Harms H. 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ Sci Technol 38: 228A-231A

Sexton K, Linder SH (2011) Am J Public Health

Shoeib M., Harner T. (2002) Characterization and Comparison of Three Passive Air Samplers for Persistent Organic Pollutants, Environ. Sci. Technol., 36 (19), 4142–4151.

Sibley SD, Pedersen JA. 2008. Interaction of the macrolide antimicrobial clarithromycin with dissolved humic acid. Environ Sci Technol 42: 422-428

Sison-Young RL, Kia R, Heslop J, Kelly L, Rowe C, Cross MJ, Kitteringham NR, Hanley N, Park BK, Goldring CE. Human pluripotent stem cells for modelling toxicity. Adv Pharmacol; 2012; 63:207-56

Solomon KR, Brock TCM, de Zwart D, Dyer SD, Posthuma L, Richards SM, Sanderson H, Sibley PK, van den Brink PJ. 2008. Extrapolation for Criteria Setting and Risk Assessment. In: Solomon KR, Brock TCM, de Zwart D, Dyer SD, Posthuma L, Richards SM, Sanderson H, Sibley PK, van den Brink PJ, editors. Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals. Pensacola, FL, USA: CRC/Taylor and Francis/SETAC Press. p 1-32.

Song MY, Stark JD, Brown JJ 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environmental Toxicology and Chemistry 16 (12):2494-2500

Stark JD (2010) Development and use of matrix population models for estimation of toxicant effects in ecological risk assessment. In: Thorbek P, Forbes VE, Heimbach F, Hommen U, Thulke H-H, Van den Brink PJ, Wogram J, Grimm V, eds, Ecological Models for Regulatory Risk Assessments of Pesticides: Developing a Strategy for the Future. SETAC, Pensacola, FL, USA, pp 33–46.

Stephens ML, Barrow C, Andersen ME, Boekelheide K, Carmichael PL, Holsapple MP and Lafranconi M (2012) Accelerating the development of 21st-century toxicology: outcome of a Human Toxicology Project Consortium workshop. Toxicol Sci 125:327-334.

Stillman RA, Goss-Custard JD. 2010. Individual-based ecology of coastal birds. Biological Reviews 85: 413–434.

Stull, R.B., 1988. An Introduction to Boundary Layer Meteorology. Kluwer Academic Publishers, Dordrecht, The Netherlands

Su JG et al (2009) Environ Sci Technol 43: 7626-7634

Takikita, M, Chung JY, Hewitt SM Tissue microarrays enabling high-throughput molecular pathology. Curr Opin Biotechnol. 2007 18:318-25.

Tanneberger K, Rico-Rico A, Kramer NI, Busser FJM, Hermens JLM, Schirmer K. 2010. Effects of solvents and dosing procedure on chemical toxicity in cell-based in vitro assays. Environ Sci Technol 44: 4775-4781

Ter Laak TL, Agbo SO, Barendregt A, Hermens JLM. 2006. Freely dissolved concentrations of PAHs in soil pore water: Measurements via solid-phase extraction and consequences for soil tests. Environ Sci Technol 40: 1307-1313

Terceiro, M. 2005. J. Southern New England / Mid-Atlantic winter flounder. In: Mayo, R.K., Terceiro, M., editors. 2005. Assessment of 19 Northeast groundfish stocks through 2004. 2005 Groundfish Assessment Review Meeting (2005 GARM), Northeast Fisheries Science Center, Woods Hole, Massachusetts, 15-19 August 2005. U.S. Dep. Commer., Northeast Fish. Sci. Cent. Ref. Doc. 05-13; 499

Thibodeaux, L. J., Mackay D. 2010. Handbook of Chemical Mass Transport in the Environment, Taylor and Francis (CRC Press), 631 p

Thienemann, A., 1918. Lebensgemeinschaft und Lebensraum. Naturwiss. Wochenschr 17, 281-303

Thorbek P, Forbes V, Heimbach F, Hommen U, Thulke HH, van den Brink PJ, Wogram J, Grimm V (eds.). 2010. Ecological models for regulatory risk assessments of pesticides: developing a strategy for the future. Pensacola and Boca Raton (FL): Society of Environmental Toxicology and Chemistry (SETAC) and CRC Press.

Thulke HH, Eisinger D, Beer M. 2011. The role of movement restrictions and pre-emptive destruction in the emergency control strategy against CSF outbreaks in domestic pigs. Preventive Veterinary Medicine, 99: 28-37.

Thulke HH, Grimm V. 2010. Ecological models supporting management of wildlife diseases. In: Thorbek P, Forbes V, Heimbach F, Hommen U, Thulke HH, van den Brink PJ, Wogram J, Grimm V (eds). Ecological models for regulatory risk assessments of pesticides: developing a strategy for the future. Pensacola and Boca Raton (FL): Society of Environmental and Chemistry (SETAC) and CRC Press, pp. 67-76.

Tiede K, Boxall AB, Tear SP, Lewis J, David H, Hassellov M.. 2008. Detection and characterization of engineered nanoparticles in food and the environment, Food Addit Contam Part A Chem Anal Control Expo Risk Assess., 25(7):795-821.

TNO/RIVM 2006. Risk assessment report on zinc metal, zinc distearate, zinc oxide, zinc chloride, zinc sulphate and trizinc bis(orthophospate). Office for Official Publications of the European Communities, Luxembourg.

Tolls J. 2001. Sorption of veterinary pharmaceuticals - a review. Environ Sci Technol 35: 3397-3406

Topping CJ, Dalkvist T, Forbes VE, Grimm V, Sibly RM. The potential for the use of agent-based models in ecotoxicology. 2009. In: Devillers, J. (ed.) Ecotoxicology Modeling, Springer, pp. 205-237.

Trapp S, Franco A, Mackay D. 2010. Activity-Based Concept for Transport and Partitioning of Ionizing Organics. Environ Sci Technol 44: 6123-6129

Trapp S. 2000. Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. Pest Management Science 56: 767-778

Turco, L., Catone, T., Caloni, F., Di Consiglio, E., Testai, E., Stammati, A., 2011. Caco-2/TC7 cell line characterization for intestinal absorption: how reliable is this in vitro model for the prediction of the oral dose fraction absorbed in human? Toxicol. In Vitro 25, 13–20.

U.S. EPA 2007. U.S. Environmental Protection Agency, Science Advisory Board (SAB) Review of the Estimation Programs Interface Suite (EPI SuiteTM), EPA-SAB-07-11, http://www.epa.gov/sab/panels/epi_suite_review_panel.htm

US EPA 2012 U.S. Environmental Protection Agency, ECOTOX database, http://cfpub.epa.gov/ecotox

Usseglio-Polatera P, Bournaud M, Richoux P, Tachet H. 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biol 43: 175-205

Valet G (2005). Cytomics, the human cytome project and systems biology: top-down resolution of the molecular biocomplexity of organisms by single cell analysis. Cell proliferation 38, 171-174.

Van Aggelen, G., Ankley, G.T., Baldwin, W.S., Bearden, D.W., Benson, W.H., Chipman, J.K., Collette, T.W., Craft, J.A., Denslow, N.D., Embry, M.R., et al. 2010. Integrating omic technologies into aquatic ecological risk assessment and environmental monitoring: hurdles, achievements, and future outlook. Environmental Health Perspectives 118: 1-5.

Van den Brink PJ, Verboom J, Baveco JM, Heimbach F .2007. An individual-based approach to model spatial population dynamics of invertebrates in aquatic ecosystems after pesticide contamination. Environ Toxicol Chem 26: 2226–2236

Van den Brink, P.J. (2008). Ecological risk assessment: from book-keeping to chemical stress ecology. Environ. Sci. Technol. 42: 8999 – 9004.

Van den Brink, P.J., Alexander, A., Desrosiers, M., Goedkoop, W., Goethals, P., Liess, M. & Dyer, S.D. (2011b). Traits-based Approaches in Bioassessment and Ecological Risk Assessment – Strengths and weaknesses. Integrated environmental assessment and management. 7, 198–208.

Van den Brink, P.J., Rubach, M.N., Culp, J.M., Pascoe, T., Maund, S.J. & Baird, D.J. (2011a). Traits-based Ecological Risk Assessment (TERA): Realising the potential of ecoinformatics approaches in ecotoxicology. Integrated Environmental Assessment and Management. 7, 169–171.

Van der Geest HG, Soppe WJ, Greve GD, Kroon A, Kraak MHS 2002. Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly Ephoron virgo. Environmental Toxicology and Chemistry 21 (2):431-436

Van der Meer, J. 2006. Metabolic theories in ecology. Trends in Ecology and Evolution 21:136–140Van Straalen NM 2003. Ecotoxicology becomes stress ecology. Environ Sci Technol 37: 325-330

Van Straalen NM 2003. Ecotoxicology becomes stress ecology. Environ Sci Technol 37: 325-330

Van Straalen, N.M., Roelofs, D. (2008) Genomics technology for assessing soil pollution. Journal of Biology 7: 19.

Van Wijngaarden, R.P.A., Arts, G.H.P., Belgers, J.D.M., Boonstra, H., Roessink, I., Schroer, A.F.W., Brock, T.C.M., 2010. The species sensitivity distribution approach compared to a microcosm study: A case study with the fungicide fluazinam. Ecotox. Environ. Safe. 73, 109-122.

Veltman K, McKone TE, Huijbregts MA, Hendriks AJ (2009). Bioaccumulation potential of air contaminants: combining biological allometry, chemical equilibrium and mass-balances to predict accumulation of air pollutants in various mammals. Toxicology and applied pharmacology 238, 47-55.

Vermeirssen ELM, Korner O, Schonenberger R, Suter MJF, Burkhardt-Holm P. 2005. Characterization of environmental estrogens in river water using a three pronged approach: Active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish. Environ Sci Technol 39: 8191-8198

Verro R, Finizio A, Otto S, Vighi M., 2009 Predicting pesticide environmental risk in intensive agricultural areas. II: Screening level risk assessment of complex mixtures in surface waters. Environ Sci Technol. 43(2):530-7.

Versteeg, D.J., Belanger, S.E., Carr, G.J., 1999. Understanding single-species and model ecosystem sensitivity: Data-based comparison. Environ. Toxicol. Chem. 18, 1329-1346.

Von Elm E., Altman D.G., Egger M., Pocock S.J., Gøtzsche P.C., and Vandenbroucke J.P., The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. The Lancet, Volume 370, Issue 9596, Pages 1453 – 1457.

Wetmore BA, Wambaugh JF, Ferguson SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ, 3rd, Dix DJ, Andersen ME, Houck KA, Allen B, Judson RS, Singh R, Kavlock RJ, Richard AM and Thomas RS (2012) Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol Sci 125:157-174.

Wiederholm, T. 1980. Use of Benthos in Lake Monitoring. J Water Pollut Control Fed 52: 537-547.

Wildgust MA, Jones MB 1998. Salinity change and the toxicity of the free cadmium ion [Cd-(aq)(2+)] to Neomysis integer (Crustacea : Mysidacea). Aquatic Toxicology 41 (3):187-192

Wright RJ (2009). Stress and acquired glucocorticoid resistance: a relationship hanging in the balance. The Journal of allergy and clinical immunology 123, 831-832

Yuille M, van Ommen GJ, Bréchot C, Cambon-Thomsen A, Dagher G, Landegren U, Litton JE, Pasterk M, Peltonen L, Taussig M, Wichmann HE, Zatloukal K. Biobanking for Europe. Brief Bioinform. 2008;9(1):14-24

Zafar MI, Van Wijngaarden RP, Roessink I, Van den Brink PJ. 2011. Effects of time-variable exposure regimes of the insecticide chlorpyrifos on freshwater invertebrate communities in microcosms. Environ Toxicol Chem. 2011 Jun;30(6):1383-94. doi: 10.1002/etc.509. Epub 2011 Apr 8.