transatlantic think tank for toxicology

t4 Report*

Analysis of the Proposed EU Regulation Concerning Biocide Products and its Opportunities for Alternative Approaches and a Toxicology for the 21st Century

Daniele Ferrario [1] *and Richard R. Rabbit* [2]

[1] Private Consultant, Varese, Italy; [2] CAAT-Europe, University of Konstanz, Germany

Summary

On June 12, 2009, the European Commission adopted a proposal for a Regulation concerning the placement on the market and use of biocidal products, which, when it enters into force on January 1, 2013, will repeal and replace Directive 98/8/EC. The main reason for the revision of the current Directive was to promote best practices for environmental and human health protection, along with implementation of current developments in safety testing in order to create safer biocides. Moreover, the proposed Regulation aims to take into consideration the newest legislation on chemicals.

This article evaluates the proposed Regulation in comparison to Directive 98/8/EC. Although the new proposal requires the sharing of vertebrate animal test data, both for product authorization and for newly developed active substances, it misses – in contrast to REACH – the opportunity to recognize the accelerating development of alternative approaches to animal testing, most recently with new momentum provided by "Toxicity Testing for the 21st Century", and to support the evolution of toxicology towards a new approach to testing. The new methods promise not only to decrease animal pain and suffering, but also to provide faster results and better prediction for human risk assessment compared to traditional methods. Unfortunately, methods mandated for human risk assessment in the proposal are still mainly based on traditional animal study extrapolation.

We put forward and discuss possible alternative strategies, such as in vitro testing, integrated testing strategies, toxicokinetics, "omics," systems biology, bioinformatics, and computational modeling, all of which could be more encouraged by the proposal. Current opportunities to improve our tools for biocide risk assessment are discussed, delineating advantages, limitations, and development needs. It is suggested to open the proposed Regulation to alternative approaches that are based on human biology more than on extrapolation from animals to humans.

Keywords: European Regulation, European Directive, biocidal products, animal testing, alternative approaches

1 Introduction

The Directive 98/8/EC of the European Parliament and Council of February 16, 1998 concerning the placement of biocidal products (or biocides) on the market (EC, 1998) establishes a harmonized regulatory framework regarding the authorization, production, and placement on the market of biocides in the European Union. This Directive establishes a two-tier system where the Community evaluates and approves active substances; thereafter, individual Member States authorize products containing these substances. The Directive also aims to establish a list of active substances responsible for the toxicity of biocides and pesticides that are allowed to be used in such products without unacceptable effects on the environment or on human or animal health (Annex I or IA).

On June 12, 2009, the European Commission adopted a proposal for a Regulation concerning the placement on the market and the use of biocidal products (EC, 2009a), which is the

*a report of t4 – the transatlantic think tank for toxicology, a collaboration of the toxicolgically oriented chairs in Baltimore, Konstanz and Utrecht sponsored by the Doerenkamp-Zbinden Foundation

subject of this article. The proposed Regulation is scheduled to enter into force on January 1, 2013 and will repeal and replace the current Directive 98/8/EC. The progress of the legislative process involving Parliament and Council is documented by the European Commission (EC)[1]. The revision aims to increase safety for both human health and the environment, as well as to eliminate weaknesses and shortcomings of the current regulatory framework. Such a revision of the Directive also should more effectively explore the technical progress and improved scientific knowledge acquired during these years. The main objective of this proposal is to improve the existing regulatory framework, without reducing the high level of protection for European consumers, animals, and environment. Moreover, the proposal also looks toward harmonizing current procedures with the legislation on chemicals, i.e. with Regulation EC 1907/2006 (REACH) (EC, 2006), as well as Regulation 1272/2008 on classification, labeling, and packaging of substances and mixtures (EC, 2008).

Under the new proposal, data requirements shall be more aligned with the actual needs of the evaluating authorities. It will become possible to waive requirements if data are not scientifically necessary or not relevant. The proposal, for the first time, aims to render mandatory the sharing of vertebrate animal test data for product authorization and for active substances, in exchange of compensation. Apart from reducing costs for applicants and producers, this specific proposal should help to reduce the number of animals used for regulatory testing approaches.

To achieve a high level of environmental and human health protection, the proposed Regulation sets out "exclusion criteria" to prevent authorization of active substances with the worst hazard profiles, including substances that can cause cancer, mutations, reproductive/developmental toxicity, and hormone-linked diseases. Moreover, where there are indications that an active substance used in biocides may pose a higher risk than previously thought, the Commission should review the use of that active substance, ensuring that only active substances with lower risks to humans, animals, and the environment will remain on the market. In the course of authorization renewal, if a comparison between current active substances and the substitute candidates shows that the latter have less hazardous profiles or side effects on humans and/or the environment compared to the former, the active substances should be replaced.

Although the proposal strives to minimize animal testing by including obligations for data sharing and by decreasing redundant vertebrate testing, it gives little incentive to move away from the traditional scheme under which companies are required to conduct animal tests for each active substance. At this point, 6,000 animals, including dogs, rabbits, and rodents, can be necessary to test a single biocidal active substance. During these tests the animals may experience pain, convulsions, and death, all without pain relief. Traditional toxicological studies form the principal approach in the proposal with minor opportunities for novel approaches to active substance testing.

In general, the new Regulation is a compromise between promoting the best practice for environmental and human health protection, simplification, animals, and cost savings. However, it also should stimulate and generate progress toward novel approaches as in many other product fields (cosmetics, drugs, chemicals), such as most progressively promoted in "Toxicity Testing for the 21st Century" (Hartung, 2009; Leist et al., 2008a). In general, the consensus on the need for laboratory animal welfare is increasing worldwide, and the new proposal takes some steps in this direction. However, there are still (psychological) barriers to implementing alternative approaches for toxicity testing. A stronger commitment to the generation of information on biocidal product evaluation and authorization by alternative means not involving tests on animals should be highly encouraged. This review aims to provide a critical appraisal of the legislation, as well as to discuss possible alternative strategies to develop safer biocides and to prevent human risk.

2 Biocides

According to the Directive 98/8/EC of the European Parliament and Council of February 16, 1998, biocidal products (or biocides) are defined as active substances and preparations containing one or more active substances that, in the form in which they are supplied to the user, are intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. Thus biocides, by definition, affect living organisms, including humans. Despite the name, a biocide does not actually have to kill; it may cause harm, repel, or control harmful organisms by chemical or biological means. Biocidal products contain or generate active substances used against harmful organisms such as pests and bacteria. Biocides are everywhere; even drinking water can be treated with chlorine, which is considered one of the most widely used industrial biocides today.

We are exposed to such a multitude of biocides every day that it is hard to quantify them. It is even harder to try to define a dose of exposure or an acceptable daily intake. More than 80,000 commercial chemicals have been identified and listed under the Toxic Substances Control Act (TSCA) (EPA, 2004) and we are exposed to a number of mixtures, metabolites, and degradation products that currently is impossible to calculate (Hartung, 2011). With regard to biocides, it is exceedingly difficult to identify the real number of substances present on the market. In 2007, a study on the impact of biocides on the market was contracted with three consultants (namely Hydrotox GmbH, Risk & Policy Analysts Ltd, Ökopol GmbH). The main scope of this study was to assess the impact of the suggested revision of Directive 98/8/EC on the market, and assess its benefits, consequences for pest control, and level of protection. Additional information was obtained from statistics, national product registers, and the scientific literature. The main conclusions from the study indicated that only limited and inconsistent statistical information on the volume and value of the European biocide market was available, so the impact of biocides on the market was difficult to estimate;

[1] http://ec.europa.eu/environment/biocides/revision.htm#revision

moreover, the statistics generally do not distinguish between biocide and pesticide active substances.

A list of 23 product types with an indicative set of descriptions defining "biocides" is available in Annex V of the Directive 98/8/EC. These include a broad range of compounds, such as household products, cosmetics, pharmaceuticals, and human hygiene products, e.g., disinfectants. They also include medical/veterinary hygiene products, repellents, and food preservatives (Tab. 1). Others are used in applications such as wood and material preservatives, anti-fouling paints, and embalming products.

Tab. 1: List of active substances used in biocide products, general usage and general mode of action

Source: Scientific Committee on Emerging and Newly Identified Health Risks. (http://ec.europa.eu/health/archive/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf)

Active substances in biocidal products	Usage/areas of applications	General mode of action
Quaternary ammonium compounds	Healthcare, household products, surface preservation, food industry, pharmaceutical/cosmetic (preservation)	Membrane destabilizer, at a high concentration – produce cytoplasmic protein aggregation (loss of tertiary structure)
Biguanides	Healthcare, household products	Chlorhexidine specifically inhibits membrane-bound ATPase.
Phenols/cresols	Healthcare, home care products, surface preservation (various applications)	Triclosan: enoyl acyl reductase at a low concentration. Dinitrophenol collapses membrane energy (ATP synthesis). A low concentration of Fentichlor and triclosan inhibits energy-dependent uptake of amino acids. A low concentration of Triclosan discharges membrane potential in *E. faecalis.*
Alcohols	Healthcare, pharmaceutical/cosmetic (preservation)	Inhibition of DNA and RNA synthesis, cell wall synthesis Low concentration of phenoxyethanol induce proton translocation in *E. coli*
Aldehydes	Healthcare, pharmaceutical/cosmetic (preservation), industry (paper)	Alkylating agents
Ethylene oxide	Healthcare, single-used medical devices (e.g., catheter sterilization)	Alkylating agent
Anionic agents	Household products, pharmaceutical/cosmetic (preservation)	As part of a formulation (i.e. usually not the main active)
Organic acids	Pharmaceutical/cosmetic (preservation), food preservation	Dissipation of PMF; Inhibition of uptake of amino acids
Metallic salts	Healthcare, pharmaceutical preservation	Interactions with thiol-group (mercury, silver)
Isothiazolinones	Personal care products, household products and industrial products	BIT (benzisothiazolnone) affects active transport and oxidation of glucose in *S. aureus*, activity of thiol-containing enzymes, ATPases, glyceraldehyde-3-phosphate dehydrogenase
Peroxides	Healthcare, personal care products and industrial products	Oxidizing agents
Chlorine compounds and halogens	Healthcare, household products, industrial products, water treatment (private and industrial use)	Oxidizing agents
Amphoteric agents	Healthcare, household products	Unknown membrane interaction
Non-ionic agents	Healthcare, household products	Unknown membrane interaction
Limonene	Household and industrial products	Unknown membrane interaction
Antimicrobial dyes	Healthcare	DNA-intercalating agents
Iodophors	Healthcare products	Covalent binding to thiol groups
Pentamidine, isethinate of pentamidine, propamidine (dibromo derivatives)	Medical devices (e.g., catheters)	Inhibition of DNA synthesis

Due to their toxic properties and wide spectrum of activity on living beings, biocides may be harmful not only to the intended targets but also to the environment and other living beings.

In the last decades there has been an increasing need to develop new biocides, first because of societal pressure to improve safety and human health, and second because of the development of pest resistance. Many biocides are persistent; they do not break down into "safer" constituent parts but rather remain intact over long periods of time and are capable of long-range transport and bioaccumulation in human and animal tissues. In fact, large parts of the US population had detectable levels of dichloro-diphenyl-trichlororthane (DDT) in fatty tissues more than a decade after DDT was banned from the US in 1972. Biocides have different modes of action and targets of toxicity. However, a full description of different classes of biocides, route of exposure, and toxic outcome is beyond the scope of this article.

Regulations usually require that all biocides and their active ingredients be individually tested, and the list of animal tests that companies may be required to conduct comes to more than two dozen. Skin and eye irritation, sensitization, developmental and reproductive toxicity, among others, as well as acute and repeated dose tests normally are required for regulatory biocide assessment. Testing a new product may consume several thousand animals, usually rodents, rabbits, fish, birds, and dogs. Results are then used to extrapolate possible toxicity to humans; species differences and especially vulnerable individuals are covered by standard safety factors.

3 Proposed Regulation versus Directive 98/8/EC: analysis of changes

The first consultation about the proposal, held on January 21-23, 2008 in Slovenia, was intended as an exchange of views and ideas between Member States and the European Commission on topics to be addressed in the revision of the Directive. Later, on April 7-8, 2008 a second consultation was held in Germany, with representatives of industry, regulatory authorities, and non-governmental authorizations. The main topics discussed were the product definition, authorization, and data requirement approach. On May 25, 2008 the European Commission in Brussels organized a consultation of stakeholders, with participation of different biocide industries, companies, consultants, and national governments.

The proposed Regulation starts off with 68 recitals. In European legislation "recitals" use non-mandatory language and explain the background as well as the aims and objectives of the legislation. They do not set out the legal bases; these are given in the articles of the legislation. In contrast, the current Directive 98/8/EC has no recitals. As has been thoroughly discussed elsewhere (Klimas and Vaiciukaite, 2008), not all Regulations contain recitals. However, where there are recitals, parties will argue over how they should be interpreted in view of the operative provisions and whether they do or do not have other legal repercussions. For the purposes of this article we selected only the recitals that seemed most appropriate regarding regulatory approaches and toxicity testing. They have been summarized below:

(10) "With view to achieving a high level of environmental and human health protection, active substances with the worst hazard profiles should not be approved for use in biocidal products except in specific situations. These should include situations when the approval is justified because of a negligible exposure of humans to the substance, public health reasons or disproportionate negative impacts of a possible non-inclusion provided no alternatives exist."

(12) The active substances in the Community list should be regularly examined to take account of developments in science and technology. Where there are serious indications that an active substance used in biocidal products may pose higher risk than previously thought, the Commission should be able to review an inclusion of the active substance.

(13) Active substances can, on basis of their intrinsic hazardous properties, be designated as candidates for substitution with other active substances, whenever such substances considered as efficient towards the targeted harmful organisms become available in sufficient variety to avoid the development of resistances amongst harmful organisms. In order to allow for a regular examination of substances identified as candidates for substitution, the inclusion period for these substances should not, even in the case of renewal, exceed ten years. Furthermore, the identification of substances which are considered as candidates for substitution should be considered as a first step of a comparative assessment.

(14) In course of the authorization or renewal of biocidal product authorization, it should be possible to compare two or more biocidal products with regard to risks posed by them and benefits accrued through their use. As a result of such a comparative assessment, authorized biocidal products containing active substances indicated as candidates for substitution could be replaced with others that present significantly less risk to health or to the environment and where there are no significant adverse economic or practical impacts. Appropriate phase-out periods should be foreseen in such cases.

(25) To ensure a harmonized application of the low-risk criteria by competent authorities, it is necessary to specify those criteria in the Regulation as far as possible. The criteria should be based on the hazard characteristics of the biocidal products and the exposure to the product associated with its use. The use of low-risk biocidal products should not lead to a high risk of developing resistance in target organisms.

(33) When biocidal products are being authorized, it is necessary to ensure that, when properly used for the purpose intended, they are sufficiently effective and have no unacceptable effect on the target organisms such as resistance, and, in the case of vertebrate animals, unnecessary suffering and pain, and have, in the light of current scientific and technical knowledge, no unacceptable effect on the environment and on human or animal health. When deciding whether a biocidal product should be authorized, due consideration should be given to the benefits from its use.

(44) In order to encourage the research and development in active substances and biocidal products, it is necessary to establish rules under which unauthorized biocidal products or active substances may be placed on the market for the purposes of research and development.

(51) It is essential to minimize the number of tests on animals and to ensure that testing should be made dependent on the purpose and use of a product. Applicants should share, and not duplicate, vertebrate animal studies in exchange for equitable compensation. In absence of an agreement on sharing of vertebrate animal studies between the data owner and the prospective applicant, the Agency should allow the use of the studies by the prospective applicant without prejudice to the decision on the compensation made by national courts. A Community register listing the contact details of the owners of such studies should be established and put at the disposal of all authorities to inform prospective applicants.

(52) The generation of information by alternative means not involving tests on animals which are equivalent to prescribed tests and test methods should also be encouraged. In addition, the adaptation of data requirements should be used to prevent unnecessary costs related to testing.

The primary comparison of the text from Directive 98/8/EC and the proposed Regulation was based on the main body of the Regulation and has been summarized in the Table 2 (For a full comparison see Table A in the supplementary data on www.altex-edition.org). For the scope of this article the most important innovations are the followings:

a) "Proposal for Regulation": The proposal aims to turn the Directive 98/8/EC into a Regulation (*Article 1*). From the definition given in Article 189 of the Treaty of Rome: "*A Regulation shall have general application, in which every Member State has to accept the same definition*," whereas "*a Directive shall be binding upon each Member State to which it is addressed regarding the result to be achieved, but it leaves to each National Authority the choice of form and methods*." The aim of this proposal is to give less autonomy in the legislation of Member States, without the needed transposition measures and implementation legislations at the national level. A Regulation will ensure uniform application of procedures and deadlines for authorization of biocide products throughout the EU.

b) "Biocidal products treated material": The scope has been extended to cover articles and materials treated with biocidal products, including furniture and textiles (*Article 3"k"*). The Regulation also will apply to active substances generated *in situ* and to biocidal products used in materials that come into contact with food (*Article 3"r"*). Under the current Directive 98/8/EC, if an article is treated with biocides in the EU, only biocidals authorized in the EU for that purpose can be used. However, if the article treated with biocides is imported from outside EU borders there is poor control over the substances it may be treated with. It is true that customs (according to Directive 67/548/EEC and later to REACH) should ask for complete documentation of all products, but often importers are unaware of some components of the product and treatments that product may have received. Apart from the current interpretation that this discriminates some industries, the implementation of the new proposal might increase safety for consumers, since unknown or even banned active substances in the EU might represent a risk for human health or the environment. The new proposal aims to authorize the production and distribution of all articles that are treated with biocides authorized in at least one Member State. Labeling requirements have also been standardized: the labeling provisions will apply equally to both European and non-European manufacturers.

c) "Data requirements": Under the proposed Regulation, all applicants shall submit a full dossier or letter of access for all biocidal products containing the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL), Predicted Environmental Concentration (PEC), and Predicted No-Effect Concentration (PNEC). A detailed and full description of the studies conducted and of the methods used, or a bibliographical reference to those methods, shall be included. However, with the new proposal it will become possible to waive requirements if the data are not scientifically necessary, are technically impossible to supply, or are not relevant (*Article 19*). In order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort. It will no longer be possible to repeat tests that have already been carried out on vertebrate animals, and information gained from such tests must be shared, in exchange for compensation (*Article 51, 52*).

d) "Exclusion criteria": The proposed Regulation sets out "exclusion criteria" to prevent authorization of active substances with very poor hazard profiles, including substances that can cause cancer, mutations, reproductive problems, and hormonal imbalances (*Article 5, 9, 17*). Biocidal products with problematic active substances will also be compared to ensure that only the products with the lower risk remain on the market (*Article 21*). The rules on comparative assessment of active substances are also modified. The proposed system will also take into consideration those active substances that still give rise to concern although they present an acceptable pattern of toxicity (listed in Annex I), i.e. substances that are flagged for substitution. These flagged substances are then compared with other available or newly developed substances intended for the same purpose; if the flagged substance presents higher risks for human health or the environment, its authorization is refused by the Member States and production is prohibited.

e) "Impacts on animal use": The proposal strives to minimize animal testing as far as possible. Vertebrate tests may not be repeated, and a new obligation to share data involving vertebrate animal tests will come into force. This means that data owners will be obliged to share their data, in exchange for fair compensation (*Article 51, 52*). Data requirements of the Directive are also modified: long term animal toxicity studies are required only when necessary, whereas data waiving has been introduced and is aimed at reducing both the number of animals used in toxicological evaluations and the costs of production.

f) "European Chemicals Agency": The Joint Research Centre will no longer be responsible for the technical and scientific support relevant for a centralized authorization system; the European Chemicals Agency (ECHA) will be in charge of technical and scientific evaluation and coordination of all the applications for inclusion of active substances in Annex I (list of active substances with requirements agreed at the Com-

munity level for inclusion in biocidal products). The agency will also play a key role in the centralized authorization of products. In the event of any disputes over mutual recognition between the Member States or the Member States and applicants, ECHA will provide the Commission with technical and scientific support (*Article 3"q", 7, 21, 51*).

g) "Costs and benefits": Compared with the existing rules, the only change involving additional costs to industry concerns the extension of its scope to include treated articles and materials. These costs will result mainly from inclusion of further active substances in Annex I and compliance with the labeling obligations. On the other hand, obligatory data sharing for vertebrate animal data and streamlining the data requirements will possibly result in significant cost savings (*Article 51, 52*).

h) "Product authorization and mutual recognition": Under the current Directive, all biocidal products are authorized at a Member State level. This will change for two types of biocidal products – biocidal products based on new active substances and low-risk biocidal products – which will have access to a Community authorization allowing them to be placed on the market throughout the Community. All other biocidal products will still be subject to national authorizations issued by Member States. There will also be further changes to the rules on mutual recognition, the process whereby an authorization in one Member State may be recognized by another Member State. Under the Regulation, it will be possible to apply either for mutual recognition of an existing authorization, or for a mutual recognition that runs in parallel with the first authorization process (*Article 33*).

Other simplifications have been proposed regarding budgetary implication and administrative procedures; however, a full comparison is not within the scope of this article.

Tab. 2: Comparison between the proposed Regulation concerning the placing on the market and use of biocidal products with the current Directive 98/8/EC

Only articles more relevant for the purpose of this paper have been described and commented on. For a full comparison see Table A in the supplementary data on www.altex-edition.org.

Changes relevant to animal testing requirements in the *Proposal for a Regulation concerning the placing on the market and use of biocidal products*
Article 1
The proposal is turned into a Regulation; it will ensure the uniform application of the new instrument throughout the EU, in particular the procedures and the deadlines for authorisation of biocide products and mutual recognition of these authorisations.
Article 3
All substances placed on the market with the intent to generate active substances are now considered biocidal products.
Directive 98/8/EC concerning Biocidal Products entered into force on May 14, 2000; a marked distinction between existing active substances from before this date and new active substances has now been made.
For the first time any substance entered into the production of goods has to be declared.
The Regulation shall have general application, every Member State has to accept the same definition. The Commission will have more power as to the authorization and use of biocidal products.
The European Chemicals Agency will be responsible for chemical evaluation and registration.
Inclusion of material that might come into contact with food, feedstuff or is a processing aid.
Article 4
Active substances are included in Annex I for maximum 10 years.
Article 5
The exclusion of substances will be more strict.
Active substances classified in accordance to REACH as carcinogen toxic to the reproduction mutagen 1A or 1B will be excluded from Annex I.
Active substances classified in accordance to REACH as toxic to the reproduction 1A or 1B will be excluded from Annex I.
Active substances classified in accordance to article 57 (f) of Regulation (EC) No 1907/2006 as endocrine disruptors will be excluded from Annex I.
Article 7
The European Chemicals Agency will be now responsible for all submission evaluations.

Article 9
Comparative assessment between substance candidates for substitution.
Particular concerns have been raised for active substances that are likely to induce Immunotoxicity, Neurotoxicity and Endocrine disruptor activity. All active substances capable of producing such patterns of toxicity are candidates for substitution with active substances showing less toxicity.
Isomers are also included in the evaluation of possible toxic active substances.
In accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging substances likely to be classified as carcinogen, mutagen or toxic for the reproductive system should be pointed to as candidate for substitution.
Article 16
Biocidal products shall not be authorized if, according to Regulation (EC) No 1272/2008, they might decrease the fertility, and damage the unborn child (reproduction category 1A or 1B)
Article 17
Provisions for PNEC and NOAEL have been added.
Concerns as to the persistence of the substance (bio-accumulation) were added referring to REACH Annex XIII of Regulation (EC) No 1907/2006.
Concerns on endocrine disruptors, immunotoxic and neurotoxic compounds have been added in accordance with Regulation (EC) No 1272/2008.
Article 19
Inclusion of “data waiving” when the information is not scientifically necessary, or when it is technically impossible to supply information.
Article 21
In the course of renewal of authorization if comparison between current active substances and candidates as substitute shows that the latter have less hazardous profiles or side effects to humans and/or environment than the former, replacement should be granted.
Article 33
Under the current Directive, all biocidal products are authorised at Member State level. This will change for two types of biocidal products: biocidal products based on new active substances and low-risk biocidal products – which will have access to a Community authorisation allowing them to be placed on the market throughout the Community.
Article 47
For the first time complete information about treated articles or materials authorized for use in the Community will be required.
The new proposal aims for authorizing the production and the distribution of all articles that are being treated with biocides in at least one Member State.
Article 51
Sharing of vertebrate animal test data in order to decrease useless animal testing will be mandatory.
ECHA should give information about the name and contact details of the owner of the information to all applicants.
Article 52
Compensation for data sharing to owners of the experimental test data will be granted.
Article 58
Classification in accordance with Regulation (EC) 1272/2008.
ANNEX 2
Test should be conducted following good laboratory practice in accordance with Directive 2004/10/EC.
Before conducting an experiment all available *in vitro* data, *in vivo* data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) shall be assessed first.
In vivo testing with corrosive substances should be avoided.

4 Current hazard assessment strategies

As expressed in a famous Cicero quote, "the safety of the people shall be the highest law," regulatory toxicology is the branch of toxicology that was developed for the protection of public health by regulating exposure to potentially harmful materials. Early regulatory attention generally focused on preventing the acute effects of chemicals, since these effects were easily observable and could be associated with exposure. During the early decades of the 20th century, qualitative understanding of adverse effects improved, mainly through observation of humans exposed to chemicals they routinely used in the workplace. This knowledge was then often successfully extrapolated to other forms of exposure in humans.

With more chemicals being synthesized and exposure escalating, a different approach to investigating human hazard assessment was needed. Animal tests were the obvious choice, with the introduction of laboratory animal research. For many years toxicology has believed *a priori* in the relevance of animal models to predict hazard – without questioning the results if the findings resemble expectations. Rodents, in particular, have been extensively used, not because they are highly predictive for human toxicity, but mostly because they are inexpensive, easy to maintain, and breed in animal facilities. Currently, much of toxicology still relies primarily on traditional whole-animal experiments, which are well accepted by the regulatory community. Animal models are fundamental tools in life sciences, but as "models," they still represent a deviation from reality. Major shortcomings of animal testing, such as species differences in vulnerability, different toxicokinetics compared to humans, in-bred strains, insufficient group sizes, lack of metabolism of xenobiotics, costs, throughput, and others have been discussed elsewhere (Coecke et al., 2006; Stevens, 2006; Hartung, 2008; Bottini and Hartung, 2009).

To maximize safety levels, toxicological studies are often designed according to a highly cautious approach in order to minimize the number of false negatives. Thus, animals are often exposed to high / maximum tolerated doses, which hardly reflect human exposure (Sumner and Stevens, 1994; Mehendale, 1995), while humans are usually exposed to low doses of chemicals over longer periods of time. Furthermore, the exposure of laboratory animals to such doses of hazardous chemicals may lead to considerable pain, distress, and even death. This strategy has been questioned, since it may lead to inaccurate predictions, along with the generation of false positives (Hoffmann and Hartung, 2005; Leist et al., 2008b). Finally, most animal tests have never been validated. It is true that in some cases they still represent the only reasonable approach, since we cannot reconstruct a living organism from a multitude of cell systems or tissues, or through computational models.

The fundamental rethinking of animal testing came in 1959 when Russell and Burch published their book "*The Principles of Humane Experimental Technique*," which introduced the 3Rs principle: whenever possible, reduce, refine, and replace animal experimentation. Russell and Burch's book had little obvious impact on thinking or practice in the early years after its publication. Nevertheless, significant changes were taking place at that time, which eventually led to widespread acceptance of the 3Rs principles and even to their use as the basis of new laws in a number of countries, especially in Europe. The 1960s saw great progress and development in molecular and cell biology, which led to the increased use and greater acceptability of non-animal techniques as fundamental to progress in the biomedical sciences. However, at the same time, expectations of greater safety for human beings and demands for greater protection of the environment were leading to a dramatic expansion of routine toxicity testing in animals, including the introduction of new testing requirements. It was only at end of the 1970s and the beginning of the 1980s that the term "alternative method" emerged, along with the harmonization of animal testing methods, which was implemented in 1982 with the realization of harmonized guidelines for the testing of chemicals by the Organisation for Economic Cooperation and Development (OECD). Another milestone in toxicology was the European laboratory animal welfare legislation of 1986, which required the use of alternatives whenever available. It also committed the European Commission and Member States to support the availability of alternative methods (EU Directive 86/609/EEC), recently revised and reinforced in Directive 2010/63/EU (EC, 2010; Hartung, 2010). The harmonization of test guidelines at an international level has significantly reduced testing in animals, with the notable exception of basic research due to the strong increase in genetically modified animal breeding and use. Regulatory toxicology was especially resistant to change, probably based on beliefs that replacing animal testing would necessarily lead to a decline in the safety of chemicals and in consumer protection. However, a discussion has emerged over the last two decades, especially in Europe, which has helped in the transition from animal to alternative test.

In the fifty-plus years since the publication of Russell and Burch's book, the 3Rs principles – reduction, refinement, and replacement – have travelled a long, hard road to get where they are now. The great advances of molecular and cell biology paved the road toward novel and reproducible tools to investigate the adverse effects of environmental agents in a more mechanistic, less expensive, and time-saving manner. Over the last three decades we have seen the success of *in vitro* and then *in silico* approaches in most areas of the life sciences. Cell culture, for example, has greatly improved, making most cells of non-tumor origin available today. Exciting perspectives are currently emerging from stem cell technologies (Bremer and Hartung, 2004; Stummann and Bremer 2008); moreover, high quality cell preparations derived from different human cell types should become available soon. We have achieved great results in a few years; the use of *in vitro* testing has increased our physiological understanding of cellular processes and has led to the identification of a number of more general pathways of cellular toxicity.

Nevertheless, we should acknowledge that *in vitro* models may have as many limitations as *in vivo* tests (Hartung, 2007),

and therefore many drawbacks still remain in applying *in vitro* systems in regulatory toxicology. One of the most critical questions asked by regulatory toxicologists is, how closely do these *in vitro* models represent the *in vivo* situation and how accurately can they predict adverse effects on human health (Hartung and Daston, 2009)? Thus, a full replacement of the use of animal testing in regulatory processes has only been possible in restricted areas such as skin corrosion and skin irritation. This critical aspect of alternatives does not mean that *in vitro* systems are not useful, but the use of *in vitro* methods in hazard assessment will need a more sophisticated approach, since the current testing strategies still leave an enormous gap between public expectation and what our tools are actually able to deliver.

A promising approach to using fewer animals and making better predictions in the mid-term is to design "integrated testing strategies." At present, the typical process is to use a default animal test and then, in some cases, to use cell culture and computer-based methods to define the mode of action of the toxin and to interpret and balance the results further. But the best opportunity to improve regulatory toxicology lies in strategies in which optimal use is first made of all existing information about a substance and structurally similar substances, and then information is gained by approaches that do not involve animal testing, leading to targeted animal testing only if necessary. The development of physiologically based biokinetic modeling (PBBK) (Bouvier et al., 2007) has increased the possibilities to provide a better interpretation of *in vitro* toxicity data for their relevance in terms of a toxic dose in the *in vivo* situation. These models are usually used to estimate the concentration that might be reached in a certain tissue, given a certain external exposure pattern (Blaauboer, 2003; Forsby and Blaauboer, 2007).

The examples given above show that toxicology and the tools to derive hazard assessments are still evolving, and the potential for the application of *in vitro* studies in regulatory toxicity risk assessment is very promising. However, we have to realize that limitations still exist and alternative approaches also need to be substantiated with more data. The generation of such data with advanced approaches might help in understanding and defining the mechanistic pathways of toxicity. Also, the improvements in technology have helped to refine toxicity screening. We have already moved from manual handling to high-throughput technologies that allow automation and repeated execution of experiments on miniaturized cell models of thousands of replicates. The success of the genomic sequencing has brought us into the "genomic" era, where our understanding of the mammalian genome and its products is expanding at an increasing rate. Lately, large-scale analysis of genes, proteins, and metabolites has become a more integrated, widespread approach in predictive and mechanistic toxicology, allowing us to move toward toxicity based on proper understanding of primary toxic mechanisms, and thus opening up replacement and reduction opportunities. It seems reasonable that this suite of technologies, along with the data analysis, bioinformatics tools, and statistical analysis that support them, will greatly help in the evolution of hazard assessment to a science based more on mechanistic understanding than on empirical observations. Efforts to map the entirety of pathways of toxicity, the Human Toxome, have started (Hartung and McBride, 2011). These promising solutions should be highly encouraged by the proposed Regulation for inclusion as soon as demonstrated to be suitable tools to investigate biocide risk assessment.

5 Alternative methods in biocide testing

As already discussed above, biocide testing is particularly animal consuming. Directive 98/8/EC states that it "*is necessary, when biocidal products are being authorized, to make sure that, when properly used for the purpose intended, they are sufficiently effective and have no unacceptable effect on the target organisms such as resistance or unacceptable tolerance and, in the case of vertebrate animals, unnecessary suffering and pain*." To this end, the Directive has called for extensive animal-based testing to evaluate both the safety and efficacy of biocidal products and their active ingredients. Although ANNEX 2 of the proposed Regulation states that "*before conducting an experiment all available in vitro data should be assessed first*," the generation of safer active substances, as well as the exclusion of worst hazard compounds (two of the main goals of the new Regulation), may in the near future require the generation of toxicity data on a large scale. Although data requirements differ somewhat for chemical versus anti-microbial agents, standard testing requirements for "*active ingredients" include many or all of the following*[2]:

- *Acute systemic toxicity in rodents and/or rabbits via oral, inhalation, and dermal routes*
- *Eye and skin irritation in rabbits*
- *Skin (and possibly respiratory) sensitization in mice or guinea pigs*
- *Subchronic (40-90 day) studies in rodents and dogs via one or more exposure routes*
- *Chronic (12-24 month) feeding studies in rodents and dogs*
- *Lifetime (18-24 month) carcinogenicity studies in rats and mice*
- *Mutagenicity and genotoxicity studies of at least two varieties*
- *Reproductive toxicity in at least two generations of rodents*
- *Prenatal developmental toxicity in rodents and rabbits*
- *Metabolism, toxicokinetics, and dermal absorption studies in rodents*
- *Acute aquatic toxicity to fish*

In addition, each formulated biocidal product is required to undergo separate efficacy and acute toxicity testing via the oral, dermal, and inhalation routes; skin and eye irritation; and skin sensitization (known as the acute toxicity "six-pack") for labeling purposes. The following studies may also be conditionally required for chemical ingredients and/or formulated products:

- *Neurotoxicity studies in hens*
- *Immunotoxicity studies in rodents*
- *Studies in livestock and/or companion animals*
- *Partial or full life-cycle toxicity in fish*

[2] http://www.alttox.org/ttrc/eu/institutions/dg-environment.html, last accessed October 24, 2011

– *Aquatic bio-concentration in fish*
– *Acute and/or dietary toxicity to birds*
– *Reproductive toxicity to birds*
– *Toxicity to terrestrial vertebrates other than birds*

Recognized testing methods include the internationally harmonized OECD Test Guidelines, as well as methods published in Council Regulation (EC) No 440/2008.

Given the extent of animal use in toxicity testing, it was expected that the proposed Regulation might have implemented possible alternative strategies to decrease the amount of testing required for regulatory purposes. Unfortunately, the proposal mainly addresses issues regarding the simplification and adaptation of the scope of the Directive 98/8/EC, simplifications of the data protection rules and improvement of the simplified procedures, including only some mandatory data-sharing that should help save money and animal lives. It is true that data sharing might decrease animal testing more than is possible via any alternative methods. Data sharing also has the potential for significant benefits to applicants for product authorization, in terms of reduced costs for data generation. There would also be benefits to owners of data, who would gain some return on their costs for data generation.

Annex 2 of the proposed Regulation, states that "*Before new tests are carried out to determine the properties listed in this Annex, all available* in vitro *data,* in vivo *data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) shall be assessed first. In vivo testing with corrosive substances at concentration/dose levels causing corrosivity shall be avoided. Prior to testing, further guidance on testing strategies should be consulted in addition to this Annex*." This statement must be stressed, along with a broader discussion on feasibility and acceptance of alternative methods for the proposed Regulation. In fact, despite the discussion to revamp regulatory toxicology in recent years, strongly fueled by the publication of the US National Academy of Sciences and National Research Council report, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC, 2007), the need for a paradigm shift in the field of toxicology is not reflected (as for example it is in REACH) and the new biocide proposal rather conservatively relies on whole animal experiments.

As discussed above, much effort has been spent during the last decades on the development and acceptance of alternative approaches based on the 3Rs principle. Considerable progress in the acceptance of alternative *in vitro* methods in regulatory testing has been made, in particular, with genotoxicity and skin testing. However, in more complex fields of risk assessment such as repeated-dose toxicity, reproductive/developmental toxicity, and carcinogenicity, it is expected that the development of testing batteries (*in vitro* and *in silico*) and more predictive testing approaches will only overcome scientific obstacles and regulatory skepticism in the long term. In the short or mid-term, refinement or reduction of *in vivo* tests might, however, be more acceptable options, as seen with the embracing of refinement and reduction for acute toxicity and skin sensitization.

Although the discussed shift in regulatory toxicology is not expected to happen soon, it seems that toxicology has started to question traditional animal testing methods and is moving toward the implementation of new technologies. We witness a sincere rethinking and modernization of procedures that is taking place all over the world. The statement "*the generation of information by alternative means not involving tests on animals which are equivalent to prescribed tests and test methods should also be encouraged*" (Recital 52) does not suffice; it is necessary to move the discussion to a more concrete guidance on how and where alternative methods should be encouraged and used in regulatory approaches. We do have novel tools to improve risk assessment, but they are not included often enough in regulatory testing. More than two dozen animal replacement, reduction and refinement methods and testing strategies have been endorsed as scientifically validated and may be suitable for biocide testing (Tab. 3). Other alternatives are in the phase of validation and pre-validation and might be developed in the medium term (Tab.4) (Eskes and Zuang, 2005; Adler et al., 2011, Hartung et al., 2011). In addition, animal use in biocide testing could be reduced by reconsidering the two species paradigm, e.g., by substituting the second species in reproductive toxicology with (human) cell systems. Ceasing to require cancer tests on both rats and mice would help greatly in saving animal lives, as the lack of regulatory consequences of the mouse test has been shown (van Ravenzwaay, 2010).

Particular concerns have been raised for active substances that are likely to induce immunotoxicity, neurotoxicity, endocrine disruptor activity (Article 9, 17), reproductive toxicity, such as decreased fertility, and damage to the unborn child (Article 16). All active substances capable of producing such patterns of toxicity are candidates for substitution with active substances showing less toxicity. Again, many animal lives will be sacrificed to extrapolate results to humans with basically no information on mechanism-based toxicity. Such a Regulation based on hazard only criteria (not considering exposure levels and threshold doses necessary to exert an effect) raises concerns. While such an assumption of no safe dose level is common for genotoxic carcinogenicity, there is no scientific evidence of the absence of threshold doses for developmental and reproductive toxicity or for endocrine disruption, for which the same hazard-based approach shall now be applied.

Lately there have been some efforts in the direction of alternatives to animal testing on pesticide assessment that should be encouraged for biocide regulation. One example is the extended one-generation reproductive toxicity test: This Agricultural Chemical Safety Assessment (ACSA) committee approach begins with consideration of existing data on route of exposure, magnitude, and duration (Cooper et al., 2009). These factors would aid in refining or modifying animal testing but might also bring about wider inclusion of alternative approaches. More information is gathered on the chemistry of the compounds, human exposure scenarios, concentrations, absorption, disposition, metabolism, elimination routes, and systemic toxicity. At the same time, the fact that this new approach provides the opportunity to examine neurotoxic and immunotoxic effects without increasing the number of animals or costs makes it extremely attractive for stakeholders as well. The value of alternative testing for neurotoxicity for regulatory purposes has been discussed elsewhere (Coecke et al., 2006; Bal-Price et

Tab. 3: Overview of toxicological *in vivo* and *in vitro* testing methods accepted for regulatory purposes

Toxicological endpoint	*In vivo* methods (OECD Testing Guideline)	ECVAM validated *in vitro* methods (OECD Testing Guideline, if available)
Acute Toxicity	Acute Toxicity Oral (401, 420, 423, 425) Repeated dose toxicity study in rodents oral (28 day) (407) Repeated dose 90-day toxicity study in rodents (408) or non rodents (409) Chronic toxicity studies Dermal (402, 434) 21/28 day (410) Repeated dose 90-day toxicity study in rodents (411) Inhalation (403, 433, 436) 14 or 28 day study (412) Repeated dose 90-day toxicity study in rodents (413)	
Dermal absorption	Skin absorption (427)	Skin absorption (428) – not ECVAM validated
Skin irritation and corrosion	Acute dermal irritation/corrosion (404)	Transcutaneus electrical resistance test (TER) (430) *In vitro* skin corrosion: human skin model test (431) skin irritation
Eye irritation and corrosion	Acute eye irritation/corrosion (405)	Embryonated chicken egg (HET-CAM) Isolated bovine cornea (BCOP) Isolated chicken eye (CEET) Isolated rabbit eye (IRE)
Sensitization	Skin sensitization (406) Local lymph node assay (LLNA) (429)	
Endocrine effects	Two generation reproduction toxicity study (416) Repeated dose 28 day oral toxicity in rodents, updated with parameters for endocrine effects (407) Hershberger Uterotropic	Several *in vitro* screening tests under validation
Neurotoxicity	Delayed neurotoxicity of organophosphous substances, following acute exposure (418) Delayed neurotoxicity of organophosphous substances, 28 day repeated dose study Neurotoxicity study in rodents (424)	
Prenatal development	Prenatal developmental toxicity study (414)	Whole embryo culture (WEC) Micromass test (MM) Embryonic stem cell test (EST)
Pre- and postnatal development	Two generation reproduction toxicity study (416) Developmental neurotoxicity study (426)	
Fertility	Combined repeated dose toxicity study, with the reproduction/developmental toxicity screening test (422) Reproduction/developmental toxicity screening test (421) Repeated dose 28-day toxicity study (407, 410, 412)	

Tab. 3: Overview of toxicological *in vivo* and *in vitro* testing methods accepted for regulatory purposes (continued)

Toxicological endpoint	*In vivo* methods (OECD Testing Guideline)	ECVAM validated *in vitro* methods (OECD Testing Guideline, if available)
Fertility	Repeated dose 90-day toxicity study (408, 409, 411, 413) One generation reproduction toxicity study (415) Two generation reproduction toxicity study (416)	
Genotoxicity, mutagenicity	Mammalian erytrocyte micronucleus test (474) Mammalian bone marrow chromosomal aberration test (475) Sex-linked recessive lethal test in *Drosophila melanogaster* (477) Rodent dominant lethal test (478) Mammalian spermatogonial chromosome aberration test (483) Mouse spot test (484) Mouse heritable translocation assay (485) Unscheduled DNA synthesis (UDS) test with mammalian liver cells (486)	Bacterial reverse mutation test (471), not ECVAM validated *Saccharomyces cerevisiae* gene mutation assay (480), not ECVAM validated *Saccharomyces cerevisiae* mitotic recombination assay (481) , not ECVAM validated Mammalian cell gene mutation test (476), not ECVAM validated Micronucleus test (487) Sister chromatin exchange assay in mammalian cells (479), not ECVAM validated DNA damage and repair, UDS in mammalian cells (482), not ECVAM validated
Carcinogenicity	Carcinogenicity studies (451) Combined chronic toxicity /carcinogenicity studies (453)	Cells transformation assays (SHE, Balb/c 3T3, C3H10T), ECVAM prevalidated
Immunotoxicity	Repeated dose 28-day Oral Toxicity study in rodents (407)	

Tab. 4: Alternative advanced approaches for toxicity testing and their possible application in hazard risk assessment

Approach	Possible Application
High-throughput screens	Rapidly identify active compounds, antibodies or genes which modulate a particular bio-molecular pathway over a range of doses and molecular and cellular targets
Stem cell biology	Develop *in vitro* assays by using stem cells possibly of human origin produced from directed stem cell differentiation
Toxicokinetics	Identify the relationship between the systemic exposure and toxicity
Omics	Identify the pattern of genes, proteins, and metabolites involved in toxicity pathway responses
Structural Bioinformatics Approaches	Prediction and interpretation of complex multivariable data in response to an effect of perturbation or toxicity in organs, tissues or cells
Systems biology	Combine information from different cellular response pathways to better understand integrated cellular and tissue responses
Computational systems biology	Identify human exposure situations likely to provide tissue concentrations equivalent to *in vitro* activation of toxicity pathways
Quantitative structure activity relationships	Prediction of possible toxicity and metabolic pathways based on structural descriptors and chemical properties of compounds as well as comparison with other active substances
PBPK models	Identification of human exposure situations likely to provide tissue concentrations equivalent to *in vitro* concentrations leading to activation of toxicity pathways
High-content imaging	Identification of morphological perturbation at cellular and molecular level

al., 2010). At present, alternative neurotoxicity testing is performed only at the research level, since so far no *in vitro* models have been validated and accepted for regulatory purposes. The development of batteries of *in vitro* models, including the blood-brain-barrier (BBB) and embryonic stem cell test (EST), have already been discussed (Prieto et al., 2004; Leist et al., 2008b; Stummann et al., 2009), and they represent promising opportunities. These methods still require improvements in the generally rather low correlation of compound distribution, protein binding, and lipophilicity between *in vivo* and *in vitro* conditions, although a battery of *in vitro* tests might help to fill the gaps. Moreover, three *in vitro* models, the embryonic stem cell test (EST), the micromass test (MM), and the rat post-implantation whole embryo culture test (WEC) have been formally validated and recommended as screening tests for developmental toxicity. These tests can only cover certain aspects of developmental toxicity and give only limited information on mammalian reproduction, however. Nevertheless, while the application of such tests for regulatory purposes is limited, they represent valuable tools for the clarification of possible mechanisms of toxicity. The ReProTect project has shown how this could be complemented to map further aspects of the reproductive cycle (Hareng et al., 2005). Alternatives to animal testing also are emerging for immunotoxicity. One of the most promising new areas is that of immunotoxicogenomics. Progress in this area was recently reviewed by Luebke et al. (2006). The strategy is to detect critical changes in the expression of immune function-related genes that might direct more extensive functional testing of chemicals. Because it is not feasible to test all chemicals for immunotoxic potential, an immunotoxicogenomics screen could serve to identify the subset of chemicals of greatest health concern resulting in further testing.

We have discussed a number of alternative approaches that have undergone the optimization and validation process to make them suitable for biocide risk assessment. Others are in the process of development, and the proposed Biocides Regulation should be opened up for the use of those methods as valuable opportunities to improve human risk assessment. There is obviously a delay of years from the time an alternative method is developed until it is accepted for regulatory purposes. For this reason, it is of fundamental importance that, even if they are not explicitly required by the Regulation, companies carrying out alternative tests need to be reassured that such an approach would be acceptable by regulatory authorities.

6 Alternative advanced approaches for developing safer active substances

From basic toxicology lessons we have learnt that the understanding and interpretation of toxicological findings requires information mainly on three areas: (1) possible routes of exposure, (2) delivery of compound to the possible target (or targets) of action, and (3) the mechanisms of action and potency of the chemical on the target tissues. If we compare the actual risk assessment approaches, we might argue that these steps usually are not addressed in current toxicological testing. In developing safer biocides the bioavailability, fate, absorption, and excretion of active substances should be considered. This information cannot be obtained either with current testing strategies based on animals or with *in vitro* testing. Integration of both testing strategies would be one of the possible options to better predict likely human toxicity, and it should be given more emphasis in the proposed Regulation.

At the moment, the challenge of developing *in vitro* methods that match the complexity of a whole living organism is beyond our capacity. The challenge ahead for the next few years requires a strong commitment from both academia and industry to move toward alternative advanced approaches that might represent an opportunity for better prediction of possible biocide toxicity. Unfortunately, the proposed Regulation is not very open to the integration of such approaches. The Strategy formulated by the NRC committee (Andersen and Krewski, 2009; NRC, 2007) has led to a new vision based on modern technologies and a more integrated approach (Hartung and Leist, 2008; Leist et al., 2008b). This new vision includes the accumulated knowledge on pathways of toxicity, "omics" technologies, image analysis, *in silico* modeling, PBPK (physiology-based toxicokinetic modeling), and QSAR (quantitative structure activity relationships). The implementation of the vision is also a unique opportunity for Europe to improve human health safety in biocide testing. With the introduction of new active substances and mixtures into an existing inventory of hundreds of biocidal substances, there is growing concern about potential human health impacts and our capacity for handling the number of chemicals to adequately assess potential human health risk assessment. The proposed Regulation should be opened up to innovations of the Tox-21c vision for regulatory toxicology. It appears that the increase in the number and complexity of regulatory programs to address potential health effects of chemicals is related to uncertainty about our current tools to assess human risks. The more we learn, the more we realize how ignorant we are about mechanisms behind the toxicity of chemicals. Therefore, we should consider whether, if the cost of animal tests and animal welfare are not sufficient to push us to abandon animal testing, the major driving force for change should be: can we do things better? Translating this question to biocides: can we better predict safety of newly developed active substances?

As discussed above, the potential application of alternative strategies to toxicological risk assessment is promising, but the limitations are clear, too. The challenges ahead are enormous, and the possible ways to proceed further have stimulated an interesting discussion in toxicology. The need for greater emphasis on these promising approaches should be obvious also from a regulatory point of view. The changes in regulatory procedures for cosmetics, as well as the introduction of the REACH chemicals policy in Europe show the need for more extensive use of alternative approaches.

It is increasingly acknowledged that toxicokinetics (TK) might help risk assessment (Creton et al., 2009). It is surprising that, although TK plays a lead role in safety assessment of pharmaceuticals (Baldrick, 2003), its use in risk assessment for biocides in general is underestimated (Barton et al., 2006; Saghir et al., 2006). TK might give valuable information for the selection of appropri-

ate species and doses for toxicity testing and, through comparison, between internal dose in experimental animals and humans (OECD, 2010). The biological effects of chemicals, and therefore the derived risk for humans, are best correlated with the bioavailability of the dose rather than with the externally administered dose (Morgan, 1994; Saghir et al., 2006). This holds especially true for biocides, since the concentration at which humans are typically exposed is in the order of μg/kg/day (MacIntosh et al., 1996). So the correction of the effects with the amount of chemical present in blood or plasma or target organs will provide more sound information, supporting the mechanistic understanding of the mode of action and possible extrapolation across genders, exposure routes, and dose levels. This approach was also encouraged for agricultural chemical safety assessment (Carmichael et al., 2006). Also, the revision of Directive 91/414/EEC concerning the placement on the market of plant protection products contains significant new requirements for generation of toxicokinetic data for most toxicological studies (EC, 2009b).

Other alternative approaches are mushrooming (Tab. 3), above all in the US where the vision of "Toxicology Testing for the 21st Century" brought about a new approach to toxicology that is based largely on new technologies, which unfortunately are quite absent in the European regulatory guidance and the new proposal. Recent advances in computational sciences and hardware, combined with equally significant developments in molecular biology, chemistry, and "omics" are providing toxicology with a powerful new toolbox. Similarly, the development of high content imaging platforms might facilitate the understanding of chemical perturbations of cell signaling pathways and morphology (Ding et al., 2004).

Biocide Regulation should take into consideration integration of such approaches for the development and regulation of new active substances. Europe should strive towards leading this new paradigm of testing, promoting the adaptation of test guidelines and strategies, such as OECD guidelines, biocides regulation, and REACH in general, to include a stronger emphasis on the use of alternative approaches in regulatory assessments.

7 Conclusions

The proposed Regulation is a commitment by the European Commission to achieve an even higher level of environmental and human health protection compared to the current Directive 98/8/EC, while decreasing the amount of animal testing by introducing mandatory data sharing. The reduction of administrative burden, along with obligatory and very much appreciated animal data sharing for existing substances, would help in saving costs and animal lives more than is currently possible by any alternative testing method. However, for industry, the proposed Regulation is likely to increase costs to support additional active substances and seek authorization of additional biocidal products.

The implementation of the Regulation will have positive impacts on human health and the environment, given the extension of control of products not fully regulated at present, as well as the possible replacement of the worst hazard profile products. However, without a strategy of promoting the acceptance and implementation of alternative approaches for regulatory testing, the development of new active substances, and the substitution of present biocides with other ones considered less toxic, will probably call for additional animal testing by industry. There is no precise estimate of the number of biocides to which humans are exposed. Moreover, for many of these biocides no toxicity data exist. For those where toxicity data are present, the studies are typically performed in animals exposed to high doses, with subsequent extrapolation to expected human responses at much lower doses. Therefore, the need for more integrated alternative approaches used in combination with data on structure-activity, *in silico* approaches, -omics, systems biology, and biokinetic models is urgent, both for biocide testing and for chemical testing in general.

Although the development of the use of alternative methods for hazard assessment over the last decades is strongly increasing, the proposed Regulation appears to underestimate the success of this emerging field. Some alternative approaches have been presented that might help increase consumer protection and decrease animal testing. We should keep in mind that animal tests are typically less than 60% predictive between different laboratory animal species. It is likely that the predictive capacity is even lower between laboratory animals and humans under real-life exposure. Although we have to be realistic about the prospects for full replacement, while striving for reduction and refinement we should at least keep legislation open for the integration of innovative toxicological tools as soon as they emerge. Notably, integrated testing strategies (ITS) and a number of provisions to include alternative approaches have been introduced into the REACH legislation and the testing guidelines for industry. It is highly advisable that the Biocide Regulation follows a similar path.

To conclude, we suggest modifying the proposed Regulation, opening it up for the innovations arising from our rapidly evolving understanding of systems biology, kinetics, molecular biology, bioinformatics, and other computational tools that provide the potential to evaluate the effects of large numbers of biocides at concentrations relevant to human exposure levels. With the new revised Directive 2010/63/EU for the protection of laboratory animals, Europe firmly holds the leading role in promoting alternative methods based on the 3Rs, although there is still a certain reluctance to embrace the latest technological developments in specific product regulation.

References

Adler, S., Basketter, D., Creton, S., et al. (2011). Alternative (non-animal) methods for cosmetics testing: current status and future prospects – 2010. *Arch. Toxicol. 85*, 367-485.

Andersen, M. E. and Krewski, D. (2009). Toxicity testing in the 21st century: bringing the vision to life. *Toxicol. Sci. 107*, 324-330.

Baldrick, P. (2003). Toxicokinetics in preclinical evaluation. *Drug Discov. Today 8*, 127-133.

Bal-Price, A. K., Hogberg, H. T., Buzanska, L., and Coecke, S. (2010). Relevance of in vitro neurotoxicity testing for regulatory requirements: challenges to be considered. *Neurotoxicol. Teratol. 32*, 36-41.

Barton, H. A., Pastoor, T. P., Baetcke, K., et al. (2006). The acquisition and application of absorption, distribution, metabolism, and excretion (ADME) data in agricultural chemical safety assessments. *Crit. Rev. Toxicol. 36*, 9-35.

Blaauboer B. J. (2003). Biokinetic and toxicodynamic modelling and its role in toxicological research and risk assessment. *ATLA 31*, 277-281.

Bottini, A. A. and Hartung, T. (2009). Food for thought … on economic mechanisms of animal testing. *ALTEX 26*, 3-16.

Bouvier d'Yvoire, M., Prieto, P., Blaauboer, B. J., et al. (2007). Physiologically-based Kinetic Modelling (PBK Modelling): Meeting the 3Rs Agenda. The report and recommendations of ECVAM Workshop 63. *ATLA 35*, 661-671.

Bremer, S. and Hartung, T. (2004). The use of embryonic stem cells for regulatory developmental toxicity testing in vitro – the current status of test development. *Curr. Pharm. Des. 10*, 2733-2747.

Carmichael, N. G., Barton, H. A., Boobis, A. R., et al. (2006). Agricultural chemical safety assessment: A multisector approach to the modernization of human safety requirements. *Crit. Rev. Toxicol. 36*, 1-7.

Coecke, S., Ahr, H., Blaauboer, B. J., et al. (2006). Metabolism: a bottleneck in in vitro toxicological test development. The report and recommendations of ECVAM Workshop 54. *ATLA 34*, 49-84.

Cooper, R. L. (2009). Current developments in reproductive toxicity testing of pesticides. *Reprod. Toxicol. 28*, 180-187.

Creton, S., Billington, R., Davies, W., et al. (2009). Application of toxicokinetics to improve chemical risk assessment: implications for the use of animals. *Regul. Toxicol. Pharmacol. 55*, 291-299.

Ding, G., Jiang, Q., Zhang, L. J., et al. (2004). Multiparametric ISODATA analysis of embolic stroke and rt-PA intervention in rat. *Neurol. Sci. 223*, 135-143.

Eskes, C. and Zuang, V. (2005). Alternative (non-animal) methods for cosmetics testing: current status and future prospects. *ATLA 33*, 47-81.

EPA (2004). Toxic Substances Control Act as amended through P.L. 108-201, February 24, 2004. Title I – control of toxic substances.

EC (1998). Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:123:0001:0063:EN:PDF

EC (2006). Regulation (EC) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=oj:l:2006:396:0001:0849:en:pdf

EC (2008). Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF

EC (2009a), (COM (2009)267). Proposal for a Regulation of the European Parliament and of the Council concerning the placing on the market and use of biocidal products. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:DKEY=496597:EN:NOT

EC (2009b). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF

EC (2010). Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF

Food Additives Amendment to Food, Drug and Cosmetic Act of 1958 (1958). Public Law 85-929, 85th Cong., September 6, 1958.

Forsby, A. and Blaauboer, B. (2007). Integration of in vitro neurotoxicity data with biokinetic modelling for the estimation of in vivo neurotoxicity. *Hum. Exp. Toxicol. 26*, 333-338.

Hareng, L., Pellizzer, C., Bremer, S., et al. (2005). The integrated project ReProTect: a novel approach in reproductive toxicity hazard assessment. *Reprod. Toxicol. 20*, 441-452.

Hartung, T. (2007). Food for thought ... on cell culture. *ALTEX 24*, 143-152.

Hartung, T. (2008). Food for thought … on animal tests. *ALTEX 25*, 3-9.

Hartung, T. and Leist, M. (2008). Food for thought ... on the evolution of toxicology and the phasing out of animal testing. *ALTEX 25*, 91-102.

Hartung, T. (2009). Toxicology for the twenty-first century. *Nature 460*, 208-212.

Hartung, T. and Daston, G. (2009). Are in vitro tests suitable for regulatory use? *Toxicol. Sci. 111*, 233-237.

Hartung, T. (2010). Comparative analysis of the revised Directive 2010/63/EU for the protection of laboratory animals with its predecessor 86/609/EEC – a t^4 report. *ALTEX 27*, 285-303.

Hartung, T. (2011). From alternative methods to a new toxicology. *Eur. J. Pharm. Biopharm. 77*, 338-349.

Hartung, T. and McBride, M. (2011). Food for thought ... on mapping the human toxome. *ALTEX 28*, 83-93.

Hartung T, Blaauboer GJ, Bosgra S, et al. (2011). An expert consortium review of the EC-commissioned report "Alterna-

tive (Non-Animal) Methods for Cosmetics Testing: Current Status and Future Prospects – 2010". *ALTEX 28*, 183-209.

Hoffmann, S. and Hartung, T. (2005). Diagnosis: toxic! – trying to apply approaches of clinical diagnostics and prevalence in toxicology considerations. *Toxicol. Sci. 85*, 422-428.

Klimas, T. and Vaiciukaite, T. (2008). The law of recitals in European Community Legislation. *ILSA Journal of International & Comparative Law 15*. http://ssrn.com/abstract=1159604

Leist, M., Bremer, S., Brundin, P., et al. (2008a). The biological and ethical basis of the use of human embryonic stem cells for in vitro test systems or cell therapy. *ALTEX 25*, 163-190.

Leist, M., Hartung, T., and Nicotera, P. (2008b). The dawning of a new age of toxicology. *ALTEX 25*, 103-114.

Luebke, R. W., Holsapple, M. P., and Ladics, G. S. (2006). Immunotoxicogenomics: the potential of genomics technology in the immunotoxicity risk assessment process. *Toxicol. Sci. 94*, 22-27.

MacIntosh, D. L., Spengler, J. D., Ozkaynak, H., et al. (1996). Dietary exposures to selected metals and pesticides. *Environ. Health Perspect. 104*, 202-209.

Mehendale, H. M. (1995). Toxicodynamics of low level toxicant interactions of biological significance: inhibition of tissue repair. *Toxicology 28*, 251-266.

Morgan, T. (1994). Clinical pharmacokinetics and pharmacodynamics of carvedilol. *Clin. Pharmacokinet. 26*, 335-346.

OECD (2010). OECD guideline 417 for the testing of chemicals: Toxicokinetics. http://www.oecd-ilibrary.org/docserver/download/fulltext/9741701e.pdf?expires=1320478536&id=id&accname=freeContent&checksum=81AC626E4256DD3CE12B1AE98AB3496D)

NRC (2007). *Toxicity testing in the 21st century: a vision and a strategy*. Washington, DC: National Academy Press.

Prieto, P., Blaauboer, B. J., de Boer, A. G., et al. (2004). Blood-brain barrier in vitro models and their application in toxicology. The report and recommendations of ECVAM Workshop 49. European Centre for the Validation of Alternative Methods. *ATLA 32*, 37-50.

Russell, W. M. S. and Burch, R. L. (ed.) (1959). *The Principles of Humane Experimental Technique*. Wheathampstead, UK: Universities Federation for Animal Welfare.

Saghir, S. A., Mendrala, A. L., and Bartels. M. J. (2006). Strategies to assess systemic exposure of chemicals in subchronic/chronic diet and drinking water studies. *Toxicol. Appl. Pharmacol. 211*, 245-260.

Stevens, J. L. (2006). Future of toxicology – mechanisms of toxicity and drug safety: where do we go from here? *Chem. Res. Toxicol. 19*, 1393-1401.

Stummann, T. C. and Bremer, S. (2008).The possible impact of human embryonic stem cells on safety pharmacological and toxicological assessments in drug discovery and drug development. *Curr. Stem Cell Res. Ther. 3*, 118-131.

Stummann, T. C., Hareng, L., and Bremer, S. (2009). Hazard assessment of methylmercury toxicity to neuronal induction in embryogenesis using human embryonic stem cells. *Toxicology 257*, 117-126.

Sumner, D. D. and Stevens, J. T. (1994). Pharmacokinetic factors influencing risk assessment: saturation of biochemical processes and cofactor depletion. *Environ. Health Perspect. 102*, 13-22.

van Ravenzwaay, B. (2010). Initiatives to decrease redundancy in animal testing of pesticides. *ALTEX 27*, 159-161.

Weselak, M., Arbuckle, T. E., Wigle, D. T., and Krewski, D. (2007). In utero pesticide exposure and childhood morbidity. *Environ. Res. 103*, 79-86.

Correspondence to
CAAT-EU
University of Konstanz
Universitaetsstr. 10
78464 Konstanz, Germany
phone: +49 7531 882233
e-mail: caat-eu@uni-konstanz.de