

The REACH concept and its impact on toxicological sciences

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Abstract

Currently, comprehensive toxicological data are available only for a small percentage of the 30,000 substances produced in volumes of 1–100 tons per year in the EU. Substances with inadequate safety data sets may pose a risk to employees, consumers and the environment. To improve this unsatisfactory situation the European Commission put forward a draft concept that will probably become law in 2006. The acronym of this concept is REACH standing for Registration, Evaluation and Authorization of Chemicals. The aim of REACH is to systematically evaluate the risk of approximately 30,000 chemical substances produced, used or imported in quantities of 1–100 tons per year. From a practical point of view the testing requirements for these chemicals are one of the most important parts of the REACH proposal. The latter progressively increase with the volume of chemical substances, including, e.g. acute, subchronic and chronic toxicity tests. Without doubt REACH will provide an important contribution to health protection for workers and consumers. But perhaps even more importantly, REACH offers an opportunity to optimize and innovate testing strategies for chemicals. Such novel techniques are in particular RNA expression profiling, proteome analysis and metabonomics to describe alterations in gene or protein expressions patterns or in metabolite concentrations in response to toxic stimuli. Promising data have been published indicating that these techniques might identify hepato- or nephrotoxic compounds or even carcinogens differentiating between genotoxic and non-genotoxic substances. However, so far only a relatively small number of selected typical substances with well known toxic mechanisms has been tested. Therefore, the most promising innovative techniques should be optimized and validated by investigating a series of other typical but also untypical substances. In a further step a supplementary research program to REACH should be launched including promising innovative techniques (e.g. genomics, proteomics, metabonomics) but also other alternative methods (e.g. in vitro or QSAR), concentrating on the same substances that have to be tested by conventional animal studies in the mandatory part of REACH. In the present review we summarize key features of REACH, and discuss possibilities for the development of improved techniques and integrated strategies for toxicity testing.

1. Introduction

Presently, approximately 100,000 chemical substances are available on the market of the EU (European Inventory of Existing Chemical Substances, EINECS; EU, 2002). Almost a third of them are produced in volumes of 1-100 tons per year. Solid toxicological data are available only for a small percentage of these substances. Especially for chemicals marketed before 1981 there is a lack of safety data. Therefore, risks for employees, consumers and the environment cannot be assessed comprehensively. To improve this unsatisfactory situation the European Commission submitted a draft concept that is expected to become law in 2006. The acronym of this concept is REACH standing for Registration, Evaluation and Authorization of Chemicals (SRU, 2005). The aim of REACH is to systematically evaluate the risk of approximately 30,000 chemical substances produced, used or imported in quantities of 1-100 tons per year. The burden of proof of the safety of chemicals will be imposed on the manufacturers and fabricators.

The ambitious REACH concept has been discussed controversially. The proposed directive has been criticized as being bureaucratic and costly and having a negative impact on the competitiveness of chemical companies in the EU. Animal welfare groups criticize the concept because it will generate a transient but strong increase in animal experiments. On the other hand, data developed under REACH may offer a unique chance to develop and validate techniques and methods that predict toxicity faster and more precisely than the conventional techniques. In the present review we describe the key features of REACH and suggest strategies how an integrated scientific research programme could allow an enormous progress in toxicological sciences.

2. Key features of the REACH concept

The REACH concept is aimed at bringing all chemical substances produced in volumes of more than one tonne per year under a single regulatory regime. REACH does not differentiate between new and already existing chemicals. The first phase, Registration, involves submission of a technical dossier of information about the substance to a new organisation, the European Chemicals Agency (ECA). The required data depend on the volume of production or import (overview: Table 1). The submitted data will be reviewed in an Evaluation procedure to check whether they are compliant with requirements. The evaluation procedure will lead to the decision whether further tests are required. Authorization will be necessary for those chemicals that are carcinogenic, mutagenic, toxic for reproduction, very persistent or very bioaccumulating. During the authorization procedure decisions will be made, whether specific safety instructions and measures should be installed to protect human health and the environment.

From a practical point of view the detailed compilation of the required tests is one of the most important parts of the REACH proposal (Table 1). The test requirements progressively increase with the volume of the specific chemical substance. For instance acute toxicity tests are required for substances produced (or imported) between 10 and 100 tonnes per year, subchronic toxicity tests (90 days) for substances between 100 and 1000 tonnes per year and chronic toxicity/carcinogenicity studies for substances >1000 tonnes per year. Clearly, the REACH concept will cause a transient increase in animal experiments in the years following its introduction. It has been estimated that approximately 7.5-45 million experimental animals, mainly rats and mice, will be needed within the first 15 years (BfR, 2004). Afterwards the need for experimental animals would strongly decrease.

3. Improvement of health protection for workers and consumers

Currently, complete toxicological data sets and a detailed assessment of possible health risks are available only for a small percentage of the 30,000 substances produced in volumes of 1-100 tons per year. These untested substances may pose a risk to the consumer or the employee getting in contact with them. It is a major purpose of REACH to improve this unsatisfactory situation. An important feature is that REACH does not differentiate between new and existing chemicals. Obviously, the toxicity of a chemical will not depend on its date of introduction. In addition, REACH will require prior authorization for any use of particular classes of harmful chemicals, such as carcinogens, mutagens, reproductive toxicants or persistent substances. REACH is based on the precautionary principle, since the manufacturer is responsible for demonstrating the safety of his chemicals by performing appropriate toxicological studies. A further progress of REACH is seen in its authorization criterion as to whether alternative, less toxic substances are available. This is expected to lead to replacement of

hazardous chemicals by less toxic substances, e.g. in cases where chemical reactivity is not needed for the intended use. However, perhaps one of the most important longer term advantages of REACH is that it is likely to stimulate those working in the toxicological sciences to develop techniques that predict toxicity reliably and more efficiently than the conventional tests.

Table 1

Tests required by the REACH concept

Tonnage of production per yeara

Required testsb

Changes in the conceptc

<1

Not covered by REACH

≥1 to <10

Irritation/corrosion in vitro

Short-term toxicity on daphnia

No tests for most compounds

Tests required only for some compoundsd

Plus toxicity, acute

Plus biodegradation

Plus growth of algae

≥10 to <100

Eye/skin irritation, in vivo sensitisation, local lymph node acute toxicity, oral (acute toxicity inhalation, dermal) subacute toxicity (28 days), mutagenicity, bacteria cytogenicity, mammalian cells gene mutation, mammalian cells (reprotoxicity, development), (reprotoxicity, 2-generation study), short-term toxicity on daphnia, short-term growth of algae, degradation, biotic, abiotic

No test on reprotoxicity

≥100 to <1000

Eye/skin irritation, in vivo sensitisation, local lymph node (toxicokinetics) acute toxicity, oral (acute toxicity inhalation, dermal) subacute toxicity (28 days), subchronic toxicity (90 days), mutagenicity,

bacteria cytogenicity, mammalian cells gene mutation, mammalian cells reprotoxicity, development reprotoxicity, 2-generation study, toxicity on daphnia (21 days), short-term growth of algae, acute toxicity on fish, toxicity on fish (life-cycle test), degradation, biotic, abiotic sedimentation, bioaccumulation, effect on earthworm, acute effect on microorganism, growth of plants

≥1000

Eye/skin irritation, in vivo sensitisation, local lymph node (toxicokinetics) acute toxicity, oral (acute toxicity inhalation, dermal) subacute toxicity (28 days), subchronic toxicity (90 days), mutagenicity, bacteria cytogenicity, mammalian cells gene mutation, mammalian cells (carcinogenesis) reprotoxicity, development reprotoxicity, 2-generation study, toxicity on daphnia (21 days), short-term growth of algae, acute toxicity on fish, toxicity on fish (life-cycle test), degradation, biotic, abiotic sedimentation, bioaccumulation effects on earthworm, long-term effects on microorganism, effects on invertebrates, birds, plants

Italics in parenthesis indicate optional tests that are required when data indicate a risk.

- a) Per producer or importer.
- b) According to the EU Commission, October 2003.
- c) According to the consolidated draft of the Council of the European Union.
- d) Compound which meet the criteria defibed in ANNEX (Ic).

4. A unique chance to establish new methods for prediction of toxicity

Following an initiative in 1986, the EU institutions adopted in 2003 the 7th Amendment Directive 2003/15/EC on cosmetics, which aims at stepwise phasing out experimental animal safety tests of cosmetics. Activities in this and similar programmes are based on the RRR (or 3R's) principles of replacement, reduction and refinement of tests carried out using laboratory animals, principles that were first introduced by Russel and Burch (1959). As a consequence of the EU initiative, in 1991 the European Centre for the Validation of Alternative Methods (ECVAM) was founded which has become a unit of the Joint Research Centre of the EU Commission in Ispra, Italy since 1992. On an international level a close cooperation between ECVAM other similar institutions exists such as the US Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) but increasingly also with the OECD (IHCP, 2004; Zuang and Hartung, 2005; Abbot, 2005).

As already mentioned REACH is expected by many parties to cause a strong increase in animal experiments. Considerable additional activities in favour of the development and use of alternative methods will be generated by (i) avoiding expensive animal safety tests by the enterprises if possible and (ii) by the REACH proposal itself that requires use of alternative methods taking precedence over animal safety tests. Both will provide a stimulus to work towards replacement or refinement of animal experiments and towards reduction of the number of experimental animals needed to

demonstrate the safety or risks of chemicals. However, new tests must be optimized, standardized and validated before they can be accepted by regulatory authorities instead of conventional animal studies. In the past these procedures normally took several years (Zuang and Hartung, 2005). Although several promising innovative techniques are available, they have not yet been sufficiently validated with regard to their use in safety assessment. Therefore, at least in the initial phase following the introduction of REACH many substances will have to be tested in conventional animal experiments. Apart from their use in the registration of chemicals, the resulting data could be extremely useful in the establishment of new safety evaluation paradigms. Towards this goal, an integrated publicly funded research program supplementary to REACH is required. Many techniques have been recommended (Hartung et al., 2003; Gennari et al., 2004; Zuang and Hartung, 2005) that could be included into such a scientific research program. Here we summarize some methods we consider to be among the most promising:

4.1. Quantitative structure-activity relationships modelling (QSAR)

QSAR modelling is based on correlations between the chemical structure of a compound and its biological effects. Commercially available computer-based systems have already been demonstrated to allow predictions of endpoints such as mutagenicity or local tolerance to some degree (Simon-Hettich et al., 2005). However, prediction of more complex endpoints, such as acute toxicity, chronic toxicity, carcinogenicity, reproductive toxicity, or target organ toxicity is much more difficult to achieve. The further development of QSAR modelling depends, among other things, on the availability of a sufficiently large database of high quality. Data generated under REACH using standardized test methods and in accordance with GLP guidelines should be of great value for further improvement of computer-based prediction systems.

4.2. In vitro tests

Generally accepted validated alternative in vitro techniques are available for a limited number of endpoints, such as phototoxicity, skin and eye corrosion/irritation but also for some endpoints of developmental toxicity. Steered by ECVAM 10 toxicological in vitro tests have been validated up to now and six of them are meanwhile accepted by EU authorities. Further about 40 in vitro tests are being prevalidated or in the phase of validation comprising different toxicological key areas such as topical toxicity, acute and chronic systemic toxicity, sensitization, carcinogenicity, reproductive toxicity, toxicokinetics and acute fish toxicity. Development, prevalidation and validation of further techniques and methods are ongoing according to established procedures (e.g. Hartung et al., 2004; IHCP, 2004; Zuang and Hartung, 2005). Selectivity and specificity of developed or established in vitro tests may be even improved as soon as the database of substances is extended by data obtained by conventional tests according to REACH.

4.3. Genomics, proteomics and metabonomics

Recently, several scientific groups reported on the use of whole genome transcriptional profiling or proteomic analysis by two-dimensional gel electrophoresis to identify substance specific alterations in mRNA or protein expression patterns. Furthermore, NMR spectra of biofluids from experimental animals evaluated by chemometric analysis can be employed to describe variations in the pattern of metabolites following treatment with test compounds (Lindon et al., 2005). These techniques are

very promising with respect to the construction of databases for prediction of toxicity based upon the mentioned patterns of changes. Accordingly it has been reported that gene array analysis allows differentiation between test substance induced gene expression patterns associated with different subtypes of hepatotoxicity, such as microvesicular lipidosis, hepatocellular necrosis, inflammation, hepatitis, bile duct hyperplasia and fibrosis (Huang et al., 2004; Waring et al., 2001).

A further very interesting approach is the identification of patterns of gene expression deregulation specific for carcinogens. Recently, Ellinger-Ziegelbauer et al. (2005) exposed rats to the four non-genotoxic hepatocarcinogens methapyrilene, diethylstilbestrol, Wy- 14643 and piperonylbutoxide and the four genotoxic carcinogens 2-nitrofluorene, dimethylnitrosamine, NNK and aflatoxin B1 for up to 14 days and identified substance specific alterations in gene expression patterns. For instance the genotoxic carcinogens induced predominantly genes belonging to the categories DNA damage response, apoptosis and survival signalling. The nongenotoxic substances predominantly deregulated genes related to signal transduction pathways in cell cycle progression and response to oxidative DNA damage. Usually, not a single gene or pathway will be sufficient to assign a specific mechanism of carcinogenicity. But specific patterns of pathway-associated genes allowed a correct assignment of the examined substances to the groups of genotoxic or non-genotoxic rat carcinogens (Ellinger-Ziegelbauer et al., 2005).

Proteomics holds the promise for global analysis of changes in the quantities and posttranslational modifications of the proteome. Proteomic analyses are most frequently conducted by 2D gel electrophoresis for protein separation and mass spectrometry for identification. Other recently developed technologies include surface enhanced laser desorption ionization (SELDI), antibody microarrays and various types of liquid chromatography tandem mass spectrometry (LC MS/MS) (Ferguson and Smith, 2003). First successful applications could be shown in the identification of potential markers of toxicity using a proteomics approach alone (Fella et al., 2005) or in combination with other 'omics-technologies (Ruepp et al., 2002).

Recently a Consortium for Metabonomic Toxicology (COMET) generated a database of NMR spectra of biofluids from rodents treated with model toxins. This database was used to develop an expert system for prediction of target organ toxicity (mainly liver and kidney) based upon changes in biofluid metabolite profiles (Lindon et al., 2005).

The examples presented above illustrate the potential of such novel techniques to predict specific endpoints of toxicity after a short-term in vivo exposure of laboratory animals. We would like to emphasize that the information on the pathways leading to toxic effects (i.e. on the molecular mechanism of toxicity) which can be obtained from gene expression profiles and metabolite patterns will be of great value in the assessment of the relevance of alterations seen in rodents for humans. Two of the most important advantages of these methods may be the reduction of the duration of study (for instance from 2 years for carcinogenicity studies to 14 days) and of the number of required animals per dose group (for instance from 50 rodents per sex and dose group in carcinogenicity studies compared to five for gene array and metabonomic analysis). However, further research is required for establishing, standardising and validating these methods as prediction tools of specific toxic mechanisms or effects. Study conditions such as optimal species, strain, required minimum of study duration and other open questions should be thoroughly investigated. In particular the pool of

substances investigated by these methods should be extended including substances exerting weak or borderline effects or acting by atypical or unusual mechanisms

A further substantial progress would be achieved if prediction of toxicity could be based on gene expression profiles in cells instead of in vivo experiments. However, further research is required to establish cell culture conditions that guarantee an in vitro response of the transcriptome that closely resembles the in vivo situation. For instance, culture conditions for primary hepatocytes have been optimized to guarantee acceptable levels of drug metabolizing enzymes and responsiveness to enzyme inducers (Ringel et al., 2002, 2005; Carmo et al., 2005). But these conditions are not optimal to study non-genotoxic carcinogens because they do not allow a response to mitogenic stimuli equivalent to that obtained in vivo. Nevertheless, first promising results have also been obtained in vitro. For instance, aflatoxin B1, dimethylnitrosamine, acetylaminofluorene and paracetamol induced characteristic induction of transcription factors, for instance of E2F1 and Id1, in human hepatocytes (Harris et al., 2004). Furthermore, specific substance associated alterations of RNA expression patterns have been observed using cultured rat hepatocytes, liver slices and hepatocyte cell lines (de Longueville et al., 2003; Boess et al., 2003). However, comparative studies on gene expression in rat hepatocytes in vitro and in vivo indicate that the respective profiles may differ markedly. Furthermore, it should be noted that prediction of target organ toxicity cannot rely on data obtained from cells of only one organ, such as the liver.

These examples illustrate that new techniques such as gene expression profiling and metabonomics may have the potential to predict toxicologic endpoints and therefore, may be of high relevance for the future of risk assessment and risk evaluation including the REACH program. However, as already discussed these promising results have been obtained with relatively small numbers of selected substances. Presently, sensitivity and specificity of these techniques in larger batteries of substances are unknown and so far no prospective studies have been performed. Therefore, it seems unjustifiable at present to replace established animal studies, such as subchronic (90 days) toxicity tests or the 2-year carcinogenicity study by gene array analysis. However, in this respect REACH offers the unique opportunity to evaluate promising new techniques that in future could replace the tedious and more animals consuming studies.

4.4. Development of integrated testing and risk assessment strategies for chemicals

Besides development of new techniques, research should be done in order to establish improved integrated testing and risk assessment strategies. The number of already existing tests for all relevant toxicological endpoints to be considered under REACH is too high to allow an exhaustive application and validation of the complete battery of all tests and methods for all substances. Therefore, strategies have to be developed for establishing optimal combinations of already existing techniques and methods: which tests are essential after read across and QSAR considerations of a given substance, which combinations of tests individually dependent on the anticipated or already known toxicological properties of the substance could give enough information for a sound risk assessment? Research is required on the problem how to choose appropriate tests or adequate combinations of tests for a given substance and how the results from test combinations should be interpreted and be used for an optimal design of further tests if necessary (Hengstler et al., 2003; Bolt et al., 2004). Research on integrated testing and assessment strategies is a relatively young discipline that should be promoted by REACH.

5. The importance of the availability of high quality databases

REACH will generate an enormous amount of chemical and toxicological data. To make optimal use of them an efficient system for data management is required. For instance it should be possible to enter a specific chemical structure and obtain all available data on a toxicological endpoint, e.g. mutagenicity. Especially, adequate search engines for the evaluation of the complex gene array or proteomics data are needed. It should, for instance, be possible to enter a gene or a group of genes and obtain a list of substances that caused an induction of these genes. An important feature will be that the test results obtained by industry and the data from the scientific supplementary programme are made available in a single database. Without such professional data management and without integrated data quality information REACH would generate a gigantic data graveyard and gamble away the chance to improve the existing toxicological testing and risk assessment strategies. It is recognised that difficult definitions of terms and of different levels of access and information as well as difficult political decisions are required to achieve a compromise about the owners rights of a test or study and the interests of scientists or the public. However, this is considered as a task of the EU institutions to fill this gap while amending the regulation proposal.

6. Preconditions for a successful implementation of REACH

As REACH involves many different groups of interest it is understandable that the concept has been discussed controversially. However, the introduction of this new regulation should turn out to be beneficial for the further development of test methods and assessment strategies for chemicals if the following preconditions for a successful implementation of REACH will be guaranteed:

6.1. Careful validation of alternative methods replacing conventional animal studies

The REACH concept is discussed controversially also between different animal welfare groups. A frequently produced argument is that animal experiments could be replaced by in vitro techniques on a much larger scale. For instance Greenpeace principally appreciates the REACH concept, but demands an acceleration of the introduction of non-animal alternatives (Greenpeace, 2005). In principle this seems reasonable, but non-animal alternative techniques must be successfully validated before they can replace conventional animal studies (Hartung et al., 2003, 2004). This process unavoidably requires several years. A premature replacement of animal studies (for instance of those summarized in Table 1) would impair the benefits of REACH. Unless the sensitivity and specificity of non-animal alternatives have been well characterized, the premature replacement of animal studies by alternative techniques would be counterproductive for the consumers' health and block further progress in development of toxicological testing strategies.

6.2. Availability, transparency and quality of data

In order to achieve scientific progress it is necessary that the data from toxicity tests are available on different levels of information and access to the public in a transparent manner. In addition to the data, which will be submitted upon registration of chemicals, also the applied methods and if required the raw data should be available. As described above due to the large amount of data an efficient system for data management is required to allow scientific research and development of new techniques.

6.3. Scientific supplementary research program to REACH

The costs of REACH are enormous and have been estimated at 18-32 billion EUR for the period till 2020. However, these costs only comprise the mandatory part of REACH. They do not include optimization and validation of alternative techniques for toxicity testing. An adequate data management for scientific purposes is also not included. As discussed above the results obtained in the mandatory conventional toxicity tests of REACH will produce a broad database facilitating the validation of alternative techniques. Therefore, a scientific research program offers the unique chance to develop, optimise and validate new techniques and strategies for a more efficient and maybe even more reliable toxicity evaluation and an improved understanding of the involved mechanisms. It is hoped that such techniques will require much less experimental animals and less resources than those representing the state of the art today. Obviously a scientific supplementary research program would be orders of magnitude cheaper than the obligatory tests within the REACH program. The comparatively small additional costs would lead to a major improvement in the safety assessment of chemicals and thus even in Europe's competitiveness in the long run.

Acknowledgment

We thank Dr. Phil Hewitt, Head of Toxicogenomics, Institute of Toxicology, Merck KGaA, for helpful discussion.

References

Abbot, A., 2005. More than a cosmetic change. *Nature* 438, 144-146.

BfR, 2004. New EC chemicals legislation: animal welfare and consumer protection are reconcilable. From BfR homepage: <http://www.bfr.bund.de/>.

Boess, F., Kamber, M., Romer, S., Gasser, R., Muller, D., Albertini, S., Suter, L., 2003. Gene expression in two hepatic cell lines, cultured primary hepatocytes, and liver slices compared to the in vivo liver gene expression in rats: possible implications for toxicogenomics use of in vitro systems. *Toxicol. Sci.* 73, 386-402.

Bolt, H.M., Foth, H., Hengstler, J.G., Degen, G., 2004. Carcinogenicity categorization of chemicals-new aspects to be considered in a European perspective. *Toxicol. Lett.* 151, 29-41.

Carmo, H., Hengstler, J.G., de Boer, D., Ringel, M., Remiao, F., Carvalho, F., Fernandes, E., dos Reys, L.A., Oesch, F., de Lourdes Bastos, M., 2005. Metabolic pathways of 4-bromo-2,5- dimethoxyphenethylamine (2C-B): analysis of phase I metabolism with hepatocytes of six species including human. *Toxicology* 206, 75-89.

de Longueville, F., Atienzar, F.A., Marcq, L., Dufrane, S., Evrard, S., Wouters, L., Leroux, F., Bertholet, V., Gerin, B., Whomsley, R., Arnould, T., Remacle, J., Canning, M., 2003. Use of a lowdensity microarray for studying gene expression patterns induced by hepatotoxicants on primary cultures of rat hepatocytes. *Toxicol. Sci.* 75, 378-392.

Ellinger-Ziegelbauer, H., Stuart, B., Wahle, B., Bomann, W., Ahr, H.J., 2005. Comparison of the expression profiles induced by genotoxic and nongenotoxic carcinogens in rat liver. *Mutat. Res.* 575, 61-84.

EU, 2002. Corrigendum to the Commission communication pursuant to Article 13 of Council Directive 67/548/EEC of 27 June 1967 on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, as amended by Directive 79/831/EEC-EINECS (European Inventory of Existing Commercial Chemical Substances) (Off. J. Eur. Commun. C 146 A of 15.6.1990). *Off. J. Eur. Commun. C* 54, 13-73.

Fella, K., Gluckmann, M., Hellmann, I., Karas, M., Kramer, P.J., Kroger, M., 2005. Use of two-dimensional gel electrophoresis in predictive toxicology: identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. *Proteomics* 5, 1914-1927.

Ferguson, P.L., Smith, R.D., 2003. Proteome analysis by mass spectrometry. *Annu. Rev. Biophys. Biomol. Struct.* 32, 399-424.

Gennari, A., van den Berghe, C., Casati, S., Castell, J., Clemedson, C., Coecke, S., Colombo, A., Curren, R., Dal Negro, G., Goldberg, A., Gosmore, C., Hartung, T., Langezaal, I., Lessigiarska, I., Maas, W., Mangelsdorf, I., Parchment, R., Prieto, P., Sintes, J.R., Ryan, M., Schmuck, G., Stitzel, K., Stokes, W., Vericat, J.A., Gribaldo, L., 2004. Strategies to replace in vivo acute systemic toxicity testing. The report and recommendations of ECVAM Workshop 50. *ATLA-Altern. Lab. Anim.* 2, 437-459.

Greenpeace, 2005. REACH - EU chemicals policy reform; Chemicals - the proposed EU legislation, <http://eu.greenpeace.org/issues/chem.html>.

Harris, A.J., Dial, S.L., Casciano, D.A., 2004. Comparison of basal gene expression profiles and effects of hepatocarcinogens on gene expression in cultured primary human hepatocytes and HepG2 cells. *Mutat. Res.* 549, 79-99.

Hartung, T., Bremer, S., Casati, S., Coecke, S., Corvi, R., Fortaner, S., Gribaldo, L., Halder, M., Roi, A.J., Prieto, P., Sabbioni, E., Worth, A., Zuang, V., 2003. ECVAM's response to the changing political environment for alternatives: consequences of the European Union chemicals and cosmetics policies. *ATLA-Altern. Lab. Anim.* 31, 473-481.

Hartung, T., Bremer, S., Casati, S., Coecke, S., Corvi, R., Fortaner, S., Gribaldo, L., Halder, M., Hoffmann, S., Roi, A.J., Prieto, P., Sabbioni, Scott, L.E., Worth, A., Zuang, V., 2004. A modular approach to the ECVAM Principles on test validity. *ATLA-Altern. Lab. Anim.* 32, 467-472.

Hengstler, J.G., Bogdanffy, M.S., Bolt, H.M., Oesch, F., 2003. Challenging dogma: thresholds for genotoxic carcinogens? The case of vinyl acetate. *Annu. Rev. Pharmacol. Toxicol.* 43, 485-520.

Huang, Q., Jin, X., Gaillard, E.T., Knight, B.L., Pack, F.D., Stoltz, J.H., Jayadev, S., Blanchard, K.T., 2004. Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants. *Mutat. Res.* 549, 147-167.

IHCP, 2004. Institute of Health and Consumer Protection, Joint Research Centre of the EU Commission, Ispra, Piedmont, Italy. Alternative methods to animal testing. Annu. Rev., 19-26, <http://ihcp.jrc.cec.eu.int/press/activity.htm>.

Lindon, J.C., Keun, H.C., Ebbels, T.M., Pearce, J.M., Holmes, E., Nicholson, J.K., 2005. The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* 6, 691-699.

Ringel, M., Oesch, F., Gerl, M., Klebach, M., Quint, M., Bader, A., Bottger, T., Hengstler, J.G., 2002. Permissive and suppressive effects of dexamethasone on enzyme induction in hepatocyte cocultures. *Xenobiotica* 32, 653-666.

Ringel, M., von Mach, M.A., Santos, R., Feilen, P.J., Brulport, M., Hermes, M., Bauer, A.W., Schorrmann, W., Tanner, B., Schon, M.R., Oesch, F., Hengstler, J.G., 2005. Hepatocytes cultured in alginate microspheres: an optimized technique to study enzyme induction. *Toxicology* 206, 153-167.

Ruepp, S., Tonge, R.P., Shaw, J., Wallis, N., Pognan, F., 2002. Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. *Toxicol. Sci.* 65, 135-150.

Russel, W.M.S., Burch, R.L., 1959. *The Principles of Human Experimental Techniques*. Methuen, London.

Simon-Hettich, B.T., Steger-Hartmann, H., Foth, R., Kahl, P.-J., Kramer, W., Lilienblum, D., Schrenk, T., Schulz, H., 2005. Schweinfurth Stellungnahme der Beratungskommission der Gesellschaft für Toxikologie zum Einsatz von Computersystemen zur Vorhersage und Beurteilung toxischer Effekte von chemischen Stoffen. Homepage der Gesellschaft für Toxikologie, <http://www.toxikologie.de/>.

SRU (Der Rat von Sachverständigen für Umweltfragen), 2005. Die Registrierung von Chemikalien unter dem REACHRegime- Prioritätensetzung und Untersuchungstiefe.

Waring, J.F., Jolly, R.A., Ciurlionis, R., Lum, P.Y., Praestgaard, J.T., Morfitt, D.C., Buratto, B., Roberts, C., Schadt, E., Ulrich, R.G., 2001. Clustering of hepatotoxins based on mechanism of toxicity using gene expression profiles. *Toxicol. Appl. Pharmacol.* 175, 28-42.

Zuang, V., Hartung, T., 2005. Making validated alternatives available-the strategies and work of the European Centre for the validation of alternative methods (ECVAM). *AATEX-Altern. Anim. Test. Exp.* 11, 15-26.