Comment

A Critical Evaluation of the 2011 ECHA Reports on Compliance with the REACH and CLP Regulations and on the Use of Alternatives to Testing on Animals for Compliance with the REACH Regulation

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Summary — On 30 June 2011, the European Chemicals Agency published two reports, one on the functioning of the REACH system, the other on the use of alternatives to animal testing in compliance with that system. The data presented are based on information gained during the first registration period under the REACH system, which included high production volume chemicals and substances of very high concern, which have the most extensive information requirements. A total of 25,460 registration dossiers were received, covering 3,400 existing, so-called 'phase-in', substances, and 900 new, so-called 'non-phase-in', substances. Data sharing and the joint submission of data are reported to have worked successfully. In the registration dossiers for these substances, results from new animal tests were included for less than 1% of all the endpoints; testing proposals (required for 'higher-tier' information requirements) were submitted for 711 *in vivo* tests involving vertebrate animals. The registrants mainly used old, existing experimental data, or options for the adaptation (waiving) of information requirements, before collecting new information. For predicting substance toxicity, 'read-across' was the second most-used approach, followed by 'weight-of-evidence'. *In vitro* toxicity tests played a minor role, and were only used when the respective test methods had gained the status of regulatory acceptance. All in all, a successful start to the REACH programme was reported, particularly since, in contrast to most predictions, it did not contribute to a significant increase in toxicity testing in animals.

Key words: *ECHA,* in vitro *methods, (Q)SAR, REACH, read-across, regulatory toxicity, weight-of-evidence.*

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Introduction

On 30 June 2011, the European Chemicals Agency (ECHA; Helsinki, Finland) published two reports: a) on the operation of EU chemicals regulation No. 1907/2006, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, and the CLP regulation, No. 1272/2008, on the classification, labelling and packaging (CLP) of substances and mixtures (1); and b) on the use of alternatives to testing chemicals on animals in compliance with the REACH regulation (2). The submission of these two reports is a legal requirement of the REACH regulation (Article 117[2]). The REACH and CLP report presents a statistical analysis of the data submitted in 24,560 registration dossiers between 1 June 2008 and 28 February 2011. This period covers the time between the beginning of the obligation to register substances under the REACH system (1 June 2008) and the first so-called 'extended' REACH registration deadline of 30 November 2010. Since 1 June 2008, new substances, the so-called 'non-phase-in substances', require registration before manufacture or import in quantities above 1 tonne per year is allowed to begin. In contrast to these new substances, extended registration deadlines apply for the so-called phase-in (i.e. existing) substances that had been pre-registered by 1 December 2008, which form the majority of the substances to be registered up to now: existing substances manufactured or imported above 1,000 tonnes per annum (tpa) or which have special hazardous properties had to be registered by 30 November 2010. Existing substances manufactured or imported at between 100 and 1,000 tpa have to be registered by 31 May 2013,

and those imported at between 10 and 100 tpa by 31 May 2018.

As a consequence, most of the registration dossiers submitted by 28 February 2011 cover new and existing substances manufactured or imported at above 1,000 tpa. Due to their tonnage-related impact, such substances have the highest data requirements under the REACH system.

In contrast to the high number of registration dossiers submitted (24,560), the number of substances for which the registration dossiers were submitted was 4,300, with 3,400 being existing and 900 being new substances.

In order to be able to correctly interpret the outcome of the ECHA report on the use of alternatives to animal testing, the essential results of the statistical report on the operation of REACH and CLP have to be taken into account. Therefore, a short summary of the statistical report will precede the evaluation of the report on the use of alternatives.

The ECHA Report on the Operation of the REACH and CLP Regulations

Avoidance of animal testing

REACH is designed to gather data submitted in registration dossiers when companies register new substances or the existing substances. Another important principle of the REACH regulation is that new animal testing should be carried out only as a last resort (Article 25). One essential element of REACH is that it explicitly gives companies the flexibility to employ alternative, non-animal test methods and other approaches to fulfil their information requirements and to support safety claims for their substances. New animal testing is not considered scientifically necessary (Annex IX, 1), if: a) existing data can be used; b) weight-of-evidence approaches are available; c) (Q)SARs can be applied; d) suitable *in vitro* methods are available, with 'suitable' being defined as *"sufficiently well developed according to internationally agreed test development criteria (e.g. ECVAM) for the entry of a test into the pre-validation process"* (Annex IX, 1.3); or e) the grouping of substances and read-across approaches lead to the necessary information.

The basic message of the ECHA report (1) is that the REACH regulation is working well and that the various players with responsibilities are responding in a satisfactory manner. This is predominately due to the commitment and collaboration between the industry, stakeholders, the Member States, the European Commission, and the ECHA.

By the first registration deadline of 30 November 2010, 4,300 substances had been registered in 24,560 dossiers, and 3,400 of them were existing substances. These numbers are significantly lower than had been anticipated, especially when taking into account that 140,779 existing substances had been pre-registered by the deadline of 1 December 2008. Presumably, many of these pre-registrations were never intended to proceed to full registration at a later stage.

Data sharing — one substance, one registration

Data sharing, as laid down in Title III of the regulation, is one of the core principles in the REACH regulation. By submitting dossiers jointly and by sharing information on substances, companies increase the efficiency of the registration system, reduce costs, and avoid unnecessary testing on vertebrate animals. Indeed, studies involving testing on vertebrate animals *have to be shared* in any case, and new studies involving vertebrate animals *should only* be conducted if the data cannot be generated by any other means.

As a consequence, only a single dossier can be submitted for each individual substance. To achieve this goal, companies should establish a *substance information exchange forum* (SIEF) for each substance; and the registration dossier should be submitted by the lead registrant. The number of companies forming a SIEF varies considerably, e.g. SIEFs of more than 1,000 companies have been established for 146 substances. This approach to data sharing has reduced the cost of registering and testing considerably. The confidentiality and legal issues involved were unprecedented and quite challenging. In the report, the ECHA concludes that *the REACH concept of data sharing worked well and the first round of submissions was therefore a success.*

However, the complexity of substance identification for existing substances was a problem and had been underestimated. Dossier evaluation revealed that, in many registration dossiers, the substance identity of existing substances had not been described adequately. According to the ECHA's analysis, some companies reduced the number of registrations by artificially expanding the definition of a substance. As a consequence, the ECHA and the European Commission should investigate how the data-sharing procedures can be made more transparent, and how to promote best practice for data-sharing before the forthcoming registration deadlines for existing substances manufactured or imported in quantities between 10 and 1,000 tpa.

Evaluation of registration dossiers

The REACH regulation requires the ECHA to carry out *compliance checks on at least 5%* of the

total number of registration dossiers received for each tonnage band (Article 41[5]), which indicates that the REACH provisions evidently foresaw that the ECHA would have a very limited capacity to evaluate the dossiers.

Taking into account that 3,400 existing substances have been registered and that the ECHA has performed compliance checks on 5% of them, this means that only 170 of the existing chemicals have been evaluated by the ECHA, and of the 900 new substances, only 45 have been evaluated.

From the first compliance checks, the ECHA reports that *"these initial compliance checks (of 5% of the dossiers) indicate that a significant proportion of dossiers may have shortcomings and still need to be improved with further information"* and that *"the scope of the compliance check process limits the ECHA to requesting missing information."*

The ECHA Report on the Use of Alternatives to Testing in Animals

The REACH regulation requires upfront that every effort must be made to ensure that the testing of chemicals on animals is truly only undertaken as a last resort, when there is no other scientifically reliable way of determining the impact on humans or on the environment. The REACH system also demands that companies in possession of data on a chemical must share it (and must share the costs involved) with any other company that is producing the same substance, to avoid duplicate animal testing.

This report (2) is the first provided by the ECHA on the use of alternatives to testing on animals. The registration dossiers for high tonnage chemicals submitted between 1 June 2008 and 28 February 2011 were analysed, to assess the use of both animal studies and of non-animal methods.

Data sharing is the key to avoiding unnecessary animal testing, and the registration data discussed in this report show that registrants indeed have used this approach. As reported by the ECHA, the registrants also made full use of all of the non-animal alternatives available, in order to avoid testing on vertebrate animals. According to the listing of waiving possibilities presented in Annex IX of the REACH regulation, this includes not only *in vitro* studies, but also, for example, the use of existing studies or the application of non-test methods, such as (Q)SAR and read-across, to predict the properties of substances.

The report shows that, so far, very few new animal studies — 1,849 tests on vertebrate animals — were conducted for the purpose of registering existing substances. Mostly, these new tests covered the following endpoints: acute toxicity, skin sensitisation and skin or eye irritation.

Furthermore, for the higher-tier substances manufactured or imported at above 100 tpa and above 1,000 tpa, new animal testing may only be performed after submitting a testing proposal to the agency, which then has to accept the testing proposal. Between June 2008 and February 2011, the ECHA received registration dossiers for 3,308 existing and 1,347 new substances (at all tonnages). Testing proposals were made in 574 dossiers covering a total of 1,175 tests, of which 711 were vertebrate animal studies. These figures included studies on 78 substances that were submitted as category dossiers (i.e. dossiers based on read-across approaches), covering 17 chemical categories and testing proposals for 104 animal studies.

Data used in the ECHA analysis

From the original number of 24,560 registration dossiers, 17,062 were identified as being registration dossiers with a tonnage band at or above 100 tpa. Next, it was necessary to exclude dossiers for 'chemical categories' from the in-depth analysis, due to the complex endpoint inter-relationship between dossiers that so far did not permit a reliable data analysis. At or above 100 tpa, there were 568 IUCLID category dossiers (i.e. 2.3% of the total number of dossiers) covering 85 substances. (Note that IUCLID is the International Uniform Chemical Database, the key software application to submit data under the REACH system; www. iuclid.eu.)

The ECHA report concludes that these restrictions do not unduly affect the overall findings of the report, since 16,494 dossiers remained in the data set for analysis. From these 16,494 dossiers, only the 1,862 lead registrant's dossiers and dossiers for individual registrations contained endpoint information for the registered substances. Thus, the final number of dossiers available for the in-depth analyses was 1,862 existing substances. Of these, 1,504 substances are being produced at above 1,000 tpa and 218 at between 100 and 1,000 tpa. While the dossiers of all the 1,862 existing substances produced at above 100 tpa were used in the Endpoint Study Record (ESR) approach, the other approach used — the substance approach — only takes into account the 1,504 high volume chemicals produced at above 1,000 tpa.

Approaches used to analyse the use of alternatives to testing on animals

It is most important to note that the ECHA has only performed *compliance checks* on the registration dossiers, but no in-depth assessments, provided that the chosen experimental *in vivo* or *in vitro* methods or the non-testing methods, i.e. (Q)SAR, weight-of-

evidence, read-across, were appropriate. It is therefore important to note that, in a dossier, the entry of an experimental study under an endpoint does not necessarily mean that the information was indeed requested in accordance to the information requirements laid down in the REACH regulation Annexes. This is especially relevant for some endpoints, namely, repeated dose toxicity, toxicity to reproduction and developmental toxicity.

ESRs are study summary reports for a specific endpoint. For an individual substance, there can be more than one, or even many, studies for any given endpoint. In the ESR approach, all the endpoint study records submitted for all of the dossiers for a given endpoint are analysed. The ESR approach provides the overall quantitative picture of options used by registrants to provide endpoint-specific information, and thus an overview of data available for the different endpoints. However, it does not elucidate which data were actually used as key data to fulfil the information requirements, and it does not provide information on data redundancy for an individual substance.

Whereas the ESR approach is based on dossiers collectively, it is also of interest to analyse, at the substance level, how the registrants used alternative approaches. This substance approach provides the relative proportions of the principal options used by registrants to meet the information requirements per endpoint, according to the category options, namely, 'testing proposals', 'experimental studies' and 'alternative methods'. Each of these options was only counted once per endpoint for an individual substance. Therefore, the substance approach does not provide a frequency distribution on how many experimental or alternative studies have been entered per endpoint at the substance level, but it indicates which approaches were actually used to provide key data to meet the REACH information requirements.

The Use of the Endpoint Study Record (ESR) Approach

Terminology used in the ESR approach

As laid down in Annex IX of the REACH regulation, the information requirements for any given toxicological endpoint can be met by performing new experiments or by applying non-experimental methods. For the purpose of the ESR approach, the following groups of options of how registrants met the information requirements were used:

— If registrants indicated they were meeting the information requirements via *testing proposals (TP)*, this was taken as evidence that the endpoint was supposed to be obtained by future testing.

— If registrants referred to an *experimental study (ES)*, this was taken as evidence that the endpoint was obtained with experimental data. It is important to note that such studies can include both *in vivo* and *in vitro* studies, depending on which types of test method have been accepted for a given endpoint.

— If there was no ESR entry referring to an experimental study, but registrants indicated either a possibility to *omit the information* or to fill the information requirements by using *alternative approaches*, this was counted as evidence that the endpoint was obtained with an alternative method (AM). Thus, in contrast to the terminology used in the context of the Three Rs principles, the ECHA uses the term 'alternative' basically for all 'non-experimental studies'.

The ESR approach lists the following specific options for such 'non-experimental', alternative methods:

— *Read-across (RA).*

— *Data waiving of an information requirement (abbreviation FO = flags to omit)* was selected by a registrant to omit the submission of the required data by indicating that: a) testing does not appear to be scientifically necessary; b) it is technically not possible; or c) it is not necessary, based on low exposure considerations.

— *Weight-of-evidence (WE).*

— *(Q)SAR studies (QS).*

— *Miscellaneous (MS)*, which was classified by the registrant as 'Other'. Their contents cannot be further verified without detailed examination.

Acute toxicity — all routes

For many of the 1,504 dossiers covering existing substances, several studies had been performed in the past by members of the SIEF consortia. Therefore, 12,874 ESRs were submitted for this endpoint. Of these (100%), 56.9% were ES (*in vivo*), 21.4% were RA, 9.2% were FO, and 8.7% were WE.

Thus, the majority of acute toxicity ESRs were obtained from *in vivo* experimental studies. No data from *in vitro* studies or QS data were submitted for this endpoint, while 43.1% of the ESRs involved non-experimental alternatives.

Skin irritation/corrosion

Skin irritation in vitro

Validated *in vitro* methods are available for this endpoint and can be used by registrants in an inte-

grated/tiered testing strategy (ITS) according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 439 (3). For the 1,504 existing substances, 329 ESRs (100%) were submitted with information requirements having been met by the following options: 76.6% ES (*in vitro*), 11.9% RA, 0.6% FO, and 10.6% WE.

The majority of the *in vitro* skin irritation data were obtained in studies performed most recently, since the OECD only adopted a TG for *in vitro* skin irritation testing in 2010. The high percentage of experimental studies (76.6%) is remarkable, since these are true experimental non-animal studies, while the percentage of ESRs involving non-experimental alternatives is less than 25%. No QS data were submitted for this endpoint.

Skin irritation in vivo

The registrants submitted a total of 5,216 ESRs (100%) for skin irritation *in vivo* for the 1,504 existing substances. The ESR distribution analysis provided the following result: 64.1% ES (*in vivo*), 21.0% RA, 4.1% FO, and 7.7% WE.

The majority of the *in vivo* skin irritation data were obtained in studies performed before any *in vitro* skin irritation tests had been validated and accepted for regulatory purposes. No QS data were submitted, and 35.9% of the ESRs involved non-experimental alternatives.

Eye irritation

Eye irritation in vitro

To date, no validated *in vitro* method can fulfil the information requirements for this endpoint, but a positive outcome from certain *in vitro* assays, e.g. the bovine corneal opacity and permeability (BCOP) assay (OECD TG 437; 4) or isolated chicken eye (ICE) test (OECD TG 438; 5), is sufficient to classify substances as severe eye irritants. For the 1,504 existing substances, a total of 172 ESRs (100%) were submitted, with the following distribution: 86.6% ES (*in vitro*), 7.0% RA, 0.6% FO, and 2.9% WE.

The majority of *in vitro* eye irritation data were obtained in studies that were performed very recently, since validated *in vitro* eye irritation tests have been accepted by the OECD only in 2009. The high percentage of 86.6% experimental studies is remarkable, since these are true experimental non-animal studies, while the percentage of ESRs involving non-experimental alternatives was less than 15%. No QS data were submitted for this endpoint.

Eye irritation in vivo

The registrants submitted 4,221 ESRs (100%) for eye irritation *in vivo*, to fulfil the information requirements for the 1,504 existing substances. The ESR distribution analysis provided the following result: 64.3% ES (*in vivo*), 20.9% RA, 5.2% FO, and 6.6% WE.

The majority of the *in vivo* eye irritation data were obtained in studies performed before any *in vitro* eye irritation tests had been validated and accepted for regulatory purposes. No QS data were submitted, and 35.7% of the ESRs involved non-experimental alternatives.

Skin sensitisation

Skin sensitisation is the toxicological endpoint that reveals the intrinsic property of a chemical substance to cause skin sensitisation and allergic contact dermatitis in humans after repeated exposure. All of the standard skin sensitisation test methods, for which EU Total Material Requirement (TMR)/OECD TGs are available, are *in vivo* tests. They include the guinea-pig maximisation test (GPMT), the Buehler occluded patch test, and the murine local lymph node assay (LLNA, OECD TG 442A [6] and TG 442B [7]). The LLNA is considered capable of predicting the relative potency of skin sensitising chemicals, i.e. their relative power/strength to induce skin sensitisation.

Today, the LLNA is the first choice method for *in vivo* testing, and another test should only be chosen in exceptional circumstances that need to be justified. However, since the majority of data submitted by registrants were historical data, experimental studies for this endpoint comprised not only LLNA data, but also results from the GPMT and the Buehler test.

For skin sensitisation, 3,754 ESRs (100%) were submitted for the 1,504 existing substances. The ESR distribution analysis provided the following result: 55.4% ES (*in vivo*), 20.8% RA, 7.0% FO, and 13.7% WE.

The majority of ESRs involved data from *in vivo* experimental studies (55%). No QS data were submitted for this endpoint, while 44.6% of the ESRs involved data from non-experimental alternatives.

Quite unexpectedly, there were 21 entries for *in vitro* skin sensitisation studies. Further analysis revealed that, in most cases, these entries were *in vivo* LLNA tests that were misclassified by registrants as *in vitro* tests.

Repeated dose toxicity

Information on repeated dose toxicity is used to predict the effects on humans of longer-term expo-

sure to chemical substances. During the study, purpose-bred animals, usually rats or mice, receive repeated doses of a substance via the oral, dermal or inhalation routes. For all types of studies, the effects on the test animals are monitored and reported according to EU TMR/OECD TG standard protocols, to ensure that the results can be used worldwide.

There are no validated *in vitro* methods for repeated dose toxicity, and this endpoint cannot be predicted by (Q)SAR methods. The available alternative approaches are therefore mainly computational prediction methods (read-across and grouping), weight-of-evidence considerations, and omitting the studies in accordance with the requirements of the REACH regulation.

For repeated dose toxicity, 10,700 ESRs (100%) were submitted for the 1,504 existing substances. The ESR distribution analysis provided the following result: 42.1% ES (*in vivo*), 28.1% RA, 18.8% FO, 6.6% WE, and 4.4% MS.

It is surprising that less than 50% of the ESRs were from *in vivo* experimental studies. As a consequence, more than 50% of the ESRs involved data from non-experimental alternatives, mostly read-across and grouping (28.1%).

Genetic toxicity

The aim of testing for genetic toxicity (genotoxicity) is to assess the mutagenic potentials of substances, i.e. their ability to induce genotoxic effects, which may lead to cancer or cause heritable damage in humans. Information is required on the capability of substances to induce gene mutations, structural chromosome aberrations (clastogenicity), and numerical chromosome aberrations (aneugenicity). To obtain such information, many different *in vitro* and in *vivo* EU or OECD test methods are available. Non-testing options, e.g. (Q)SAR and read-across, may also provide information on the mutagenic potentials of chemical substances.

According to the REACH regulation, *in vitro* mutagenicity tests are only required for the core data set, whereas specific *in vivo* confirmatory mutagenicity studies may be necessary as higher-tier studies to be conducted after the approval of testing proposals by the ECHA.

Genetic toxicity in vitro

There were 10,322 ESRs (100%) submitted for the genetic toxicity *in vitro* endpoint for the 1,504 existing substances. The ESR distribution analysis provided the following result: 57.2% ES (*in vitro*), 22.0% RA, 3.8% FO, 12.1% WE, and 4.8% MS.

The majority of ESRs involved data from *in vitro* experimental studies (57.2%). No QS data were submitted for this endpoint, while 43.8% of the ESRs involved non-experimental alternatives. Among these, read-across accounted for 22.0% and weight-of-evidence for 12.1% of the ESRs.

Genetic toxicity in vivo

The registrants submitted 3,533 ESRs (100%) for genetic toxicity *in vivo* studies for the 1,504 existing substances. The ESR distribution showed the following pattern: 52.4% ES (*in vivo*), 24.8% RA, 6.3% FO, 11.0% WE, and 5.0% MS.

A slight majority of the ESRs involved data from *in vivo* experimental studies (52.4%), and 47.6% involved non-experimental alternatives. Among these, read-across accounted for almost 25%, while no QS data were submitted for this endpoint.

Although only *in vitro* genotoxicity data are required according to the REACH regulation, the registrants submitted existing *in vivo* genotoxicity data from their files. It is therefore probable that no new animal experiments were performed to meet the testing requirements for this endpoint.

Toxicity to reproduction

Toxicity to reproduction (screening tests and 1-generation and 2-generation studies)

Testing for reproductive toxicity is focused on two related endpoints, which are usually tested separately: a prenatal developmental toxicity study on the possible damaging effects on the developing organism, and a reproductive toxicity study covering one or more generations and analysing possible damaging effects on the ability to breed or on the development of the offspring. Both study types are essential for discovering hazards to reproduction, in order to evaluate potentially serious consequences for human reproduction, as well as for fetal and child development.

No stand-alone *in vitro* tests or computational prediction methods are currently able to predict the impact that disturbing single or multiple mechanisms could have on the entire reproductive process, including normal prenatal and post-natal development. Therefore, read-across and grouping or weight-of-evidence can be used, if scientifically justified, as justification for omitting testing for these endpoints.

For the substances produced at 10–100 tpa, a reproductive/developmental toxicity screening test (e.g. OECD TG 421 or 422) is usually required to meet the core information requirements. For substances manufactured or imported between 100–1,000 tpa, in addition to the screening study, a prenatal developmental toxicity study (according

to OECD TG 414) is usually required. For substances produced above 1,000 tpa, in addition to the lower tier tests, a two-generation reproductive toxicity study (according to OECD TG 416, EU B.35) is required.

Since, up to now, the ECHA has only performed compliance checks, it is unclear whether all the results obtained in the three *in vivo* experimental tests — the two reproductive/developmental toxicity screening tests and the two-generation reproductive toxicity study — meet the information requirements of the REACH regulation.

The registrants submitted 3,535 ESRs (100%) for the reproductive toxicity endpoint for the 1,504 existing substances. The ESR distribution was: 31.7% ES (*in vivo*), 4.2% TP (not yet decided upon), 22.8% RA, 25.6% FO, 12.1% WE and 2.5% MS.

It is surprising that less than one-third (31.7%) of ESRs for this endpoint involved *in vivo* experimental studies (with an additional 4.2% testing proposals that were not yet decided upon) and that no QS data were submitted. As a consequence, more than two-thirds (68%) of the ESRs for reproductive toxicity involved non-experimental alternatives, almost 50% of them based on waiving (25.6%) and read-across and grouping (22.1%).

However, the ECHA has not yet evaluated whether the read-across and grouping approaches for predicting the properties of substances are acceptable, whether justifications for omitting the information are in line with the REACH requirements, and whether they are adequate for the purposes of classification and labelling and/or risk assessment.

Developmental toxicity

The registrants submitted 4,217 ESRs (100%) to fulfil the information requirements for the developmental toxicity endpoint for the 1,504 existing substances. The ESR distribution analysis provided the following result: 42.3% ES (*in vivo*), 3.6% TP (not yet decided upon), 29.7% RA, 10.9% FO, 10.7% WE, 0.2% QS, and 2.6% MS.

Again, less than 50% of the documents for this endpoint involved *in vivo* experimental studies (or testing proposals), and almost no QS data were submitted. More than 50% of the ESRs involved non-experimental alternatives. Read-across and grouping of substances amounted to 30%, and waiving and weight-of-evidence to 10%, each.

Also for this endpoint, the ECHA has not yet evaluated whether the read-across and grouping approaches for predicting the properties of substances, and justifications for omitting the information, are in line with the requirements of REACH regulation, and are adequate for the purposes of classification and labelling and/or risk assessment.

Carcinogenicity

The objective of carcinogenicity studies on chemical substances is to identify potential human carcinogens, their mode(s) of action and their potency. Human data are available for only a few substances, so animal tests are generally used for detecting carcinogenic properties.

Based on the complexity and length of the process of carcinogenesis, the range of biological interactions and the many different modes of action involved, even for the same substance, it is not yet possible to obtain a full understanding and completely mimic the process by using alternative, non-animal tests. The two-year cancer assay in rodents, usually in rats or mice, is typically conducted to evaluate the cancer hazard and potency of a substance.

The registrants submitted 3,559 ESRs (100%) for the 1,504 existing substances, to fulfil the information requirements of this endpoint. The ESR distribution provided the following pattern: 38.7% ES (*in vivo*), 0.1% TP (not yet decided upon), 27.9% RA, 14.9% FO, 12.2% WE, 0.2% QS, and 6.1% MS.

Again, it is surprising that only a little more than one-third (38.7%) of the ESRs for this endpoint involved *in vivo* experimental studies, and almost no QS data were submitted. As a consequence, almost two-thirds (61.3%) of the ESRs involved non-experimental alternatives, including read-across and grouping (28%), waiving (15%) and weight-of-evidence (12%).

It is important to take into account the fact that the carcinogenicity study is not a stand-alone test, but is closely linked to genotoxicity studies. Therefore, the use of non-experimental alternatives, e.g. read-across, waiving and weight-of-evidence, is most probably based on the results from genotoxicity studies, which are primarily *in vitro* studies.

The ECHA's comment on the use of alternatives based on ESR analysis

As a general statement on the use of alternatives, the ECHA concludes that, in the context of the overall number of all ESRs taken from registration dossiers in all the tonnage bands, and both existing and new substances, *new studies represented less than 1% of the total ESRs extracted for these endpoints*.

This would imply that the registrants mainly used old (existing) experimental data as well as the options for non-experimental alternatives, including the waiving of information, before conducting new experimental *in vivo* or *in vitro* studies or submitting testing proposals to meet the information requirements of the REACH regulation.

The Substance Approach to Analysing the Use of Alternative Methods

As outlined above, the ESR approach to analysing the use of alternatives focuses on all the endpoint study reports submitted for individual endpoints, but does not provide information on the actual numbers of experimental and non-experimental studies used to meet the information requirements under the REACH regulation.

The ECHA report also presents the results of the substance approach analysis, which provides the relative proportions of the principal options used by registrants to meet the information requirements by endpoint.

Again the ECHA notes that 'experimental study' does not necessarily mean that the information requirement was met according to the requirements in the REACH Annexes. This is particularly relevant for repeated dose toxicity, toxicity to reproduction and developmental toxicity.

Acute toxicity

For acute toxicity, the requirements were met with *in vivo* experimental data in 85% of the cases, while information from using only non-experimental alternative options was provided for the remainder.

Skin irritation

The combined results of *in vitro* and *in vivo* studies used to obtain the endpoint of skin irritation per analysed substance revealed that experimental *in vitro* or *in vivo* data were provided for this endpoint in 78% of the cases, while only non-experimental alternative options were used for the other 22%.

Eye irritation

In 75% of the cases, the eye irritation endpoint requirements were met with *in vitro* or *in vivo* experimental data, while non-experimental alternative options were used in the remaining 25% of cases.

Skin sensitisation

For this endpoint, 63% of the data submitted referred to *in vivo* experimental studies, while 37% of the cases involved non-experimental alternative options.

Repeated dose toxicity

About 67% of the submitted data were from *in vivo* experimental studies, while 26% of the entries involved non-experimental alternative options, and proposals for testing were submitted for the remaining 7% of cases.

Genetic toxicity

The genetic toxicity *in vitro* endpoint was used in more than 77% of the cases, while non-experimental alternative options were used in the remaining 33%. In contrast to *in vitro* experimental data on genetic toxicity, *in vivo* data were available for over 41% of the cases. Since these were most probably historical data, this result shows that, today, this endpoint is mostly covered by *in vitro* experimental data.

Toxicity to reproduction and prenatal developmental toxicity

Specifically for these two endpoints, experimental data availability does not necessarily mean that the information requirements are fulfilled according to the REACH regulation requirements. Instead, experimental data may have been generated either by reproductive/developmental screening studies, rather than by the two specific tests for these endpoints, i.e. the two-generation study and the developmental toxicity study.

Almost 42% of the analysed existing substances submissions involved experimental *in vivo* data on toxicity to reproduction, both from screening tests and from two-generation studies, while in 48% of the cases, the registrants used non-experimental alternative options to cover the endpoint, and in 10% of the cases, proposals for new *in vivo* testing were submitted.

For prenatal developmental toxicity, experimental *in vivo* studies were available for 47% of the substances — both from screening tests and developmental toxicity tests; in 43% of the cases, non-experimental alternative options were used, and proposals for new *in vivo* testing were submitted for the remaining 10%.

Carcinogenicity

For this endpoint, the ECHA report does not provide an analysis according to the 'substance approach'.

The ECHA's Conclusions on Data Submitted to Meet the REACH Regulation Requirements

The numbers of studies involving the use of animals conducted or proposed for the purpose of

meeting the REACH regulation requirements were lower than had been expected from discussions. The reasons were that the number of high tonnage substances was lower than had been expected, data sharing between the registrants worked better than might have been expected, and the adaptation possibilities were fully used by the registrants.

In relation to the overall number of all ESRs extracted from the registration dossiers of all the tonnage bands, covering both existing and new substances, new experimental studies — both *in vitro* and *in vivo* — represented less than 1% of the total ESRs for these endpoints. Therefore, it can be concluded that registrants mainly used old experimental data, as well as the options for the adaptation of the standard information requirements and other alternatives, before electing to conduct new studies to meet their obligations and make their registration dossiers compliant with the REACH regulation.

The REACH Annexes provide a number of adaptation possibilities, which permit registrants to avoid unnecessary animal testing. The registrants made full use of these adaptation options. The data show that the registrants mainly used the results of animal studies conducted prior to the entry into force of the REACH regulation. Predicting the properties of substances by 'read-across' was the second most-common means of fulfilling the information requirements, followed by other alternative options.

The registrants submitted testing proposals for the higher-tier studies before conducting such tests. Fewer testing proposals have been submitted than had been anticipated, based on previous estimates of the availability of experimental data for the higher-tier endpoints. One reason for this appears to be that registrants used other adaptation possibilities, before resorting to making a testing proposal. Another reason is that, at least in part, registrants used the 'category' or 'read-across' approach to fill data gaps for the higher-tier studies, i.e. by proposing to conduct one study to cover more than one substance.

The report also provides the number of studies that appear to have been conducted for the purpose of compliance with the REACH regulation. The REACH Annexes IX and X require the approval of testing proposals before animal tests are conducted to fulfil the information requirements for substances imported or manufactured above 100 tpa. Nevertheless, a total of 107 studies appear to have been conducted in the absence of approved testing proposals. This will be investigated by the ECHA in future compliance checks.

The ECHA also noted that for some higher-tier test requirements, data from screening studies had been submitted, rather than data from the actual test(s) specified in the REACH regulation. If such results are found by compliance checking, the ECHA may be obliged to ask the registrant for the missing information. This may result in new animal testing, in addition to the results provided in the first ECHA report.

A focus for the compliance check over the next years will be to verify whether the read-across and grouping approaches for predicting the properties of substances, and justifications for omitting the information, are in line with the requirements of the REACH regulation, and are adequate for the purposes of classification and labelling and/or risk assessment. This has to be given special consideration in the ECHA alternatives report, since 'grouping, read-across, waiving and weight-of evidence' are the most important non-animal methods, and in particular, they have been used far more often than have *in vitro* toxicity tests.

Discussion

General aspects

During the discussions on the new EU REACH legislation for chemicals, animal welfare aspects had a high priority among legislators, the general public and the EU Commission. Therefore, the REACH regulation foresees that every effort must be made to ensure that testing chemicals on animals is undertaken only as a last resort, when there is no other scientifically reliable way of showing the impact on humans or the environment.

In his foreword to the first ECHA report on *The Use of Alternatives to Testing on Animals for the REACH Regulation 2011,* Geert Dancet, the Executive Director of the ECHA, puts special emphasis on the avoidance of animal testing: *"REACH also demands that companies in possession of data on a chemical must share it (and share the cost) with any other companies making the same substance, thereby removing the potential for duplicate testing.*"

When assessing this ECHA report, one has to take into account that the REACH regulation requires the ECHA to carry out compliance checks on *only* "at least 5%" of the total number of registration dossiers received for each tonnage band. Thus, the analysis provided is not based on an in-depth scientific analysis of the REACH registration dossiers for high production volume chemicals.

It is also important to note that, according to the REACH legislation, the dossiers are owned by the registrants, i.e. usually by industry, and that the submitters are therefore responsible for the quality of the data submitted and for the safety measures (e.g. CLP; classification, labelling and packaging) implemented. Since the ECHA only has

the competence to check the compliance and not the quality of the data submitted, there is still a high level of uncertainty about the quality of the experimental and non-experimental data that have been submitted.

In both the REACH report and the REACH and alternatives report, the ECHA uses the term 'alternatives' for all 'non-experimental' studies. Thus, in the analysis, the term 'experimental studies' is used for both *in vivo* and *in vitro* studies, and the ECHA uses the term 'alternatives' basically for 'all non-experimental' approaches to meet the REACH requirements, which includes 'read-across' and 'grouping', 'waiving', 'weight-of-evidence', and QS, as well as 'proposals for additional testing'.

Main results

From both the scientific and animal welfare perspectives, the following are the most important outcomes published in the ECHA report on alternatives to animal testing to meet the information requirements for the high production volume chemicals, which have the most extensive information requirements:

1. The industry has successfully met the unprecedented challenge of data sharing, which has significantly contributed to the avoidance of unnecessary testing in animals.
2. New experimental *in vivo* or *in vitro* studies that were performed to meet the data requirements for the REACH regulation represented less than 1% of the total ESRs.
3. Almost all data requirements for the REACH regulation were met by using existing data from the files of registrants.
4. The majority of the data submitted involved experimental studies in animals.
5. The classical experimental '*in vitro*' toxicity tests played a minor role, and were only used for a limited number of endpoints, where validated methods and OECD TGs were available, e.g. for skin and eye irritation.
6. The registrants mainly used old experimental data, as well as the options for the adaptation of the standard information requirements and other alternatives, before electing to conduct (or propose) new studies to meet their information requirements under the REACH system.
7. For predicting substance toxicity, 'read-across' was the second most-used approach followed by 'weight-of-evidence' and 'waiving'.
8. It is surprising that the results of (Q)SAR studies were almost not used at all in the dossiers, even for endpoints where this approach seems promising, such as genotoxicity and skin and eye irritation/corrosion. However, when taking into account that 'grouping' and 'read-across' are methods described in the OECD QSAR Toolbox, QSAR data have been used quite extensively.

The impact of the REACH system on testing in animals

When the REACH policy was discussed in the European Parliament, several expert groups had predicted that the new EU chemicals legislation would lead to an unprecedented increase in the number of experimental animals used for toxicity testing and, in particular, for long-term toxicity studies, e.g. systemic repeated dose toxicity, reproductive and developmental toxicity, and carcinogenicity. Even quite conservative estimates from the German Federal Institute for Risk Assessment (BfR; 8) and from the Joint Research Centre (JRC) of the EU (9), estimated the need for animal numbers between 4 and 45 million. Rovida and Hartung (10) even predicted that 54 million, and possibly even more than 100 million, research animals would be used under the REACH system, and predicted the need to register at least 68,000, but maybe even 101,000 substances, with up to 47,858 substances to be registered by the first extended registration deadline. Evidently, all such estimations did not take into account the wealth of existing information in the industry's files, or the extensive use of non-experimental estimates in the dossiers, based, for example, on 'read-across', 'weight-of-evidence' and 'waiving'.

The final numbers of existing substances registered and of new animal tests performed to meet the information requirements under the REACH system, will only be available after the third and final extended registration deadline of 31 May 2018 (1). Nevertheless, the evaluation of dossiers submitted for the first extended registration deadline, presented in the current ECHA report, already permits a more-precise picture of animal use under the REACH system, especially considering that the first extended registration deadline covers the high production volume chemicals, which require the most extensive endpoint-specific information. Furthermore, the report provides a concrete overview on the number of substances registered by 30 November 2010: 4,300 substances had been registered, 1,849 new tests on animals had been performed, and 711 testing proposals for vertebrate animal studies had been submitted. Evidently, these figures are nowhere near the enormous figures cited above.

From the point of view of animal welfare, however, the fact that there were 1,849 new toxicological tests and 711 additional testing proposals by the time of the first extended registration deadline, gives cause for concern. First, every *in vivo* toxico-

logical test implies animal distress and suffering. Furthermore, even though, undoubtedly, animal testing is still legally required for regulatory purposes, the contribution of *in vivo* test methods to ensuring human health protection is increasingly being questioned (11). Finally, the ECHA has already announced that justifications for the waiving of information requirements, or for using existing information in the registration dossiers, do not always meet the requirements laid down in the REACH regulation, meaning that further new animal testing might still be requested.

In this context, it is to be hoped that, when the quality of existing data is considered, the likelihood of obtaining relevant additional information that will actually be used to implement risk management measures when requesting new testing, will be critically evaluated. Furthermore, the European Commission is encouraged to put all possible efforts into accepting new testing and non-testing alternatives to meet the REACH information requirements without delay (such as the extended one-generation study, which was adopted as OECD TG 443 in July 2011 [12]), and to submit any requested *in vivo* test methods to an impartial assessment of their relevance and reliability for assessing human health or environmental effects.

The impact of read-across and grouping

A closer analysis of the ECHA report on alternatives shows that, so far, the most effective science-based alternative used by registrants is 'read-across and/or grouping'. As shown in Figure 1, this approach was used for the endpoints which require most animals, i.e. carcinogenicity and toxicity to reproduction. The new SAR approach was developed during the past decade through close co-operation between the Institute for Health and Consumer Protection of the EU Commission's Joint Research Centre and OECD experts; and an OECD QSAR Toolbox was made available for REACH registrants in 2008 (13). Moreover, the ECHA has actively supported the use of the new computer-based methods in several publications, e.g. in the ECHA *Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.6: QSARs and grouping of chemicals* (14), in the ECHA *Practical Guide 6: How to Report Read-across and Categories* (15), and in the ECHA *Practical Guide 10: How to Avoid Unnecessary Testing on Animals* (16).

It is encouraging to learn that the industry is applying the new tools for both hazard assessment and for CLP, and taking responsibility for risk assessment based on the non-animal methods. It is to be hoped that these new and ongoing developments will also encourage the EU Commission and the ECHA to invest in improving the QSAR Toolbox, and will ensure the maintenance of free access to these tools via the Internet.

Contribution by the industry

It has been the major goal of the REACH legislation to make the use of chemicals, both in Europe and worldwide, safer — in particular, by evaluating the toxicity data concerning existing chemicals. Since many of these existing chemicals had been in use for decades, it was obvious that there was a large amount of information in the files of the chemical industry. In essence, the REACH system aims to provide toxicity data of chemicals to regulators within a very short time-frame, in order to register, evaluate and limit exposure to the most hazardous ones. Since the registrants have co-operated and supplied existing data to the new EU chemicals agency, the ECHA, the safety of chemicals can be effectively assessed and safety measures can be implemented, while hardly any additional *in vivo* studies in animals have to be conducted. This is a major achievement, to which scientists in industry, academia and the regulatory agencies have contributed.

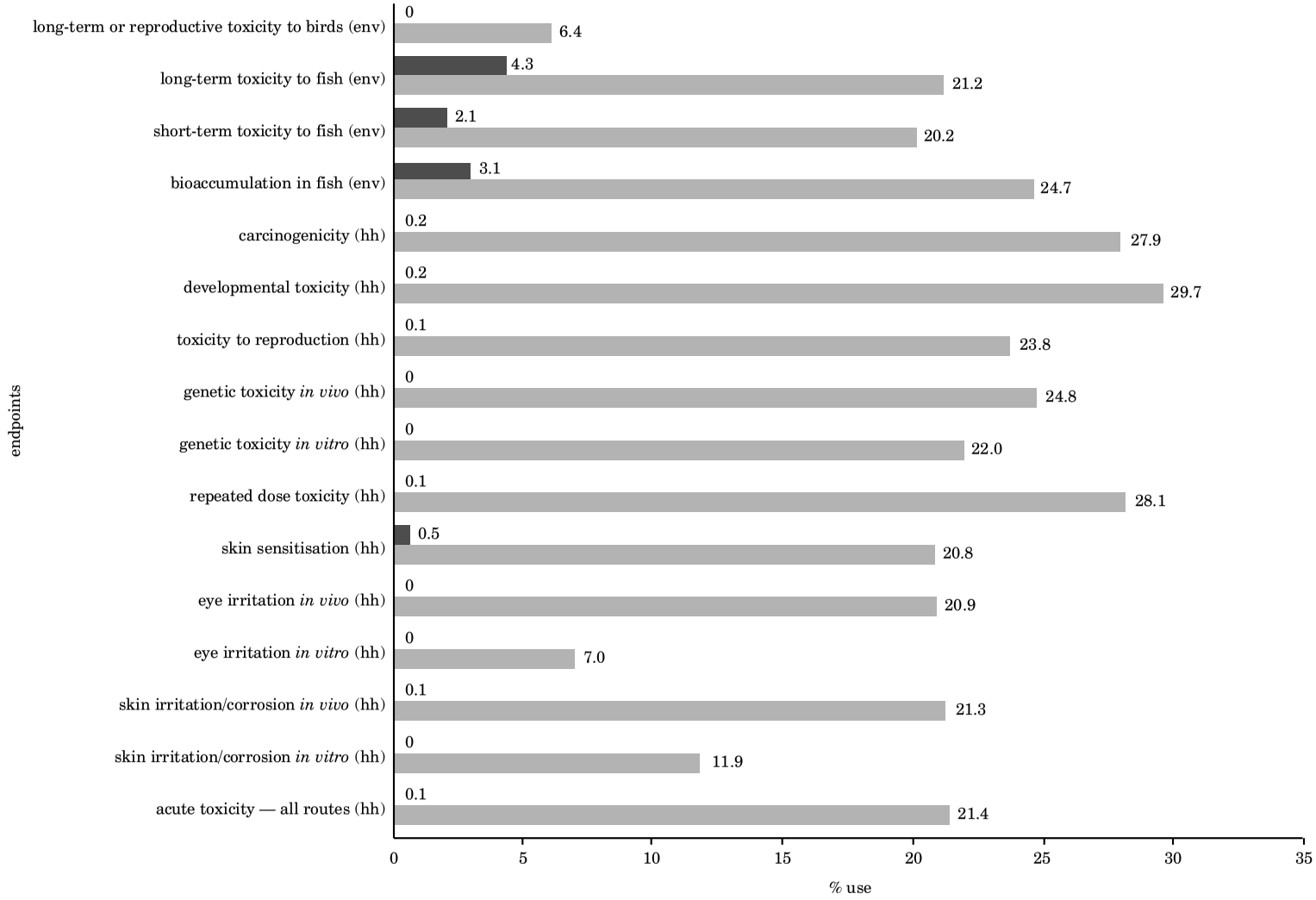
The Outlook — The REACH System as a Challenge for Ensuring Safer Chemicals in the 21st Century

The weekend before the final formal approval of the new REACH policy by the EU Parliament in 2003, the heads of the three major member states of the European Union — Tony Blair (UK), Jacques Chirac (France) and Gerhard Schroeder (Germany) — met in Berlin and came up with a very strong statement against the proposed REACH legislation. They said that it would jeopardise Europe's position in a globalised economy, since the main competitors outside Europe would not be restricted by legal requirements, and would be able to sell their chemicals at a lower cost. However, experience has shown that the REACH initiative has not provided a hurdle for Europeans, but instead, citizens and legislators outside Europe are following the European example. The REACH system represents an outstanding start for the provision of safer chemicals in the 21st century. It is now up to all those responsible to ensure that the information collected is used adequately, and that problems identified during the first registration period are addressed to further improve the application of the system for the future.

References

1. ECHA (2011). *The Operation of REACH and CLP*, 91pp. Helsinki, Finland: European Chemicals

Figure 1: QSAR and read-across methods used for submissions to the ECHA for existing substances at or above 1000 tpa



The analysis is based on details provided in the 2011 ECHA report, The Use of Alternatives to Testing on Animals for the REACH Regulation *(2).*

■ = *QSAR studies;* ■ = *read across; env = environmental endpoint; hh = human health endpoint.*

Agency. Available at: http://echa.europa.eu/doc/117reports/operation_reach_clp_2011_en.pdf (Accessed 17.10.11).
2. ECHA (2011). *The Use of Alternatives to Testing on Animals for the REACH Regulation*, 69pp. Helsinki, Finland: European Chemicals Agency. Available at: http://echa.europa.eu/doc/117reports/alternatives_test_animals_2011_en.pdf (Accessed 17.10.11).
3. OECD (2010). *OECD Guideline for the Testing of Chemicals. No. 439.* In Vitro *Skin Irritation: Reconstructed Human Epidermis Test Method*, 18pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
4. OECD (2009). *OECD Guideline for the Testing of Chemicals. No. 437. Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants*, 18pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
5. OECD (2009). *OECD Guideline for the Testing of Chemicals. No. 438. Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants*, 18pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
6. OECD (2010). *OECD Guideline for the Testing of Chemicals. No. 442A. Skin Sensitization: Local Lymph Node Assay: DA*, 16pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
7. OECD (2010). *OECD Guideline for the Testing of Chemicals. No. 442B. Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA*, 15pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
8. Höfer, T., Gerner, I., Gundert-Remy, U., Liebsch, M., Schulte, A., Spielmann, H., Vogel, R. & Wettig, K. (2004). Animal testing and alternative approaches for the human health risk assessment under the proposed new European chemicals regulation. *Archives of Toxicology* **78**, 549–564.
9. van der Jagt, K., Munn, S., Torslov, J. & de Bruijn, J. (2004). *Alternative Approaches Can Reduce the Use of Test Animals Under REACH*. JRC Report EUR 21405 EN, 31pp. Ispra, Italy: Joint Research Centre, Institute for Health and Consumer Protection. Available at: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/8790/1/EUR%2021405%20EN.pdf (Accessed 17.10.11).
10. Rovida, C. & Hartung, T. (2009). Re-evaluation of animal numbers and costs for *in vivo* tests to accomplish REACH legislation requirements for chemicals — a report by the Transatlantic Think Tank for Toxicology. *ALTEX* **26**, 187–208.
11. US National Research Council (2007). *Toxicity Testing in the 21st Century: A Vision and a Strategy*, 216pp. Washington, DC, USA: The National Academies Press. Available at: http://www.nap.edu/catalog.php?record_id=11970#toc (Accessed 17.10.11).
12. OECD (2011). *OECD Guideline for the Testing of Chemicals. No. 443. Extended One-Generation Reproductive Toxicity Study*, 25pp. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.oecd-ilibrary. org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788 (Accessed 17.10.11).
13. OECD (2008). *The OECD QSAR Toolbox*. Paris, France: Organisation for Economic Co-operation and Development. Available at: http://www.qsartoolbox.org/ (Accessed 17.10.11).
14. ECHA (2008). *Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.6: QSARs and grouping of chemicals*, 134pp. Helsinki, Finland: European Chemicals Agency. Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r6_en.pdf?vers=20_08_08 (Accessed 17.10.11).
15. ECHA (2009). *Practical Guide 6: How to Report Read-across and Categories*, 34pp. Helsinki, Finland: European Chemicals Agency. Available at: http://echa.europa.eu/doc/publications/practical_guides/pg_report_readacross_categ.pdf (Accessed 17.10.11).
16. ECHA (2011). *Practical Guide 10: How to Avoid Unnecessary Testing on Animals*, 19pp. Helsinki, Finland: European Chemicals Agency. Available at: http://echa.europa.eu/doc/publications/practical_guides/pg_10_avoid_animal_testing_en.pdf (Accessed 17.10.11).