ECHA
EUROPEAN CHEMICALS AGENCY

The Use of Alternatives to Testing on Animals for the REACH Regulation

Second report under Article 117(3) of the REACH Regulation

Europe Direct is a service to help you find answers to your questions about the European Union.

Freephone number: 00 800 6 7 8 9 10 11
Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

More information on the European Union is available on the Internet (http://europa.eu).

HOW TO OBTAIN EU PUBLICATIONS

Free publications

- via EU Bookshop (http://bookshop.europa.eu)
- at the European Union's representations or delegations. You can obtain their contact details on the Internet (http://ec.europa.eu) or by sending a fax to +352 2929-42758

Priced publications

- via EU Bookshop (http://bookshop.europa.eu)

Priced subscriptions (e.g. annual series of the Official Journal of the European Union and reports of cases before the Court of Justice of the European Union)

- via one of the sales agents of the Publications Office of the European Union (http://publications.europa.eu/others/agents/index_en.htm)

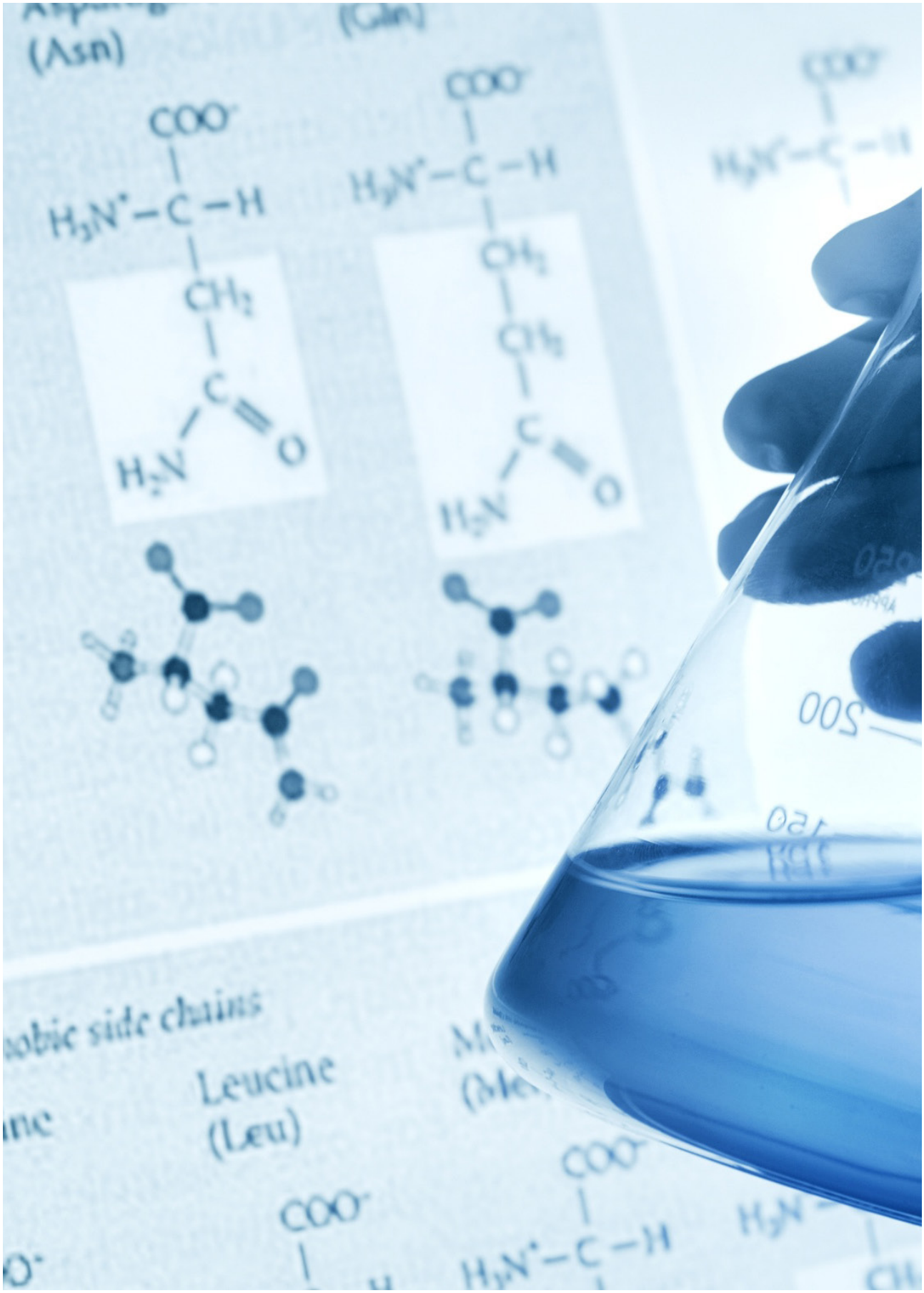
The Use of Alternatives to Testing on Animals for the REACH Regulation
Second report under Article 117(3) of the REACH Regulation

Reference: ECHA-14-A-07-EN
Cat. number: ED-04-14-483-EN-N
ISBN: 978-92-9244-593-5
DOI: 10.2823/22471
Date: 2 June 2014
Language: English

If you have questions or comments in relation to this document please send them (quoting the reference and issue date) using the information request form. The form can be accessed via the 'Contact ECHA' page at: http://echa.europa.eu/contact

European Chemicals Agency
Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland
Visiting address: Annankatu 18, Helsinki, Finland

Table of Contents


(Asn)
(Gln)
Leucine
(Leu)

Foreword by the Executive Director

Dear reader,

This is the second report that ECHA has made for the European Commission on how companies use alternatives to animal testing under the REACH Regulation. The analysis is based on data mining techniques and focuses on the numerical use of alternative methods. Dossier quality is not covered in this report – we address that issue in our annual evaluation reports and our decisions on testing proposals and compliance checks.

According to REACH, using animals for testing should be a last resort – only when there are no other scientifically reliable ways of assessing the potential effects on humans or the environment. There are a number of alternatives available: comparing substances with similar ones; grouping them together into logical categories; specialised computer modelling; bringing together a weight of evidence; and non-animal tests, for example, *in vitro* studies using cell rather than animals.

Our analysis once again shows that registrants do make use of the alternatives. The most common and widely used alternatives are building categories and predicting substance properties by "read-across". REACH also demands that companies share data with other companies that are making the same substance, thereby avoiding duplicate testing. The report shows that most registrants do comply with their obligation to share data.

Although the report is formally addressed to the European Commission, it is also of interest to many readers. To make this technical subject more accessible, we have also produced a summary leaflet, which will be available in 23 EU languages.

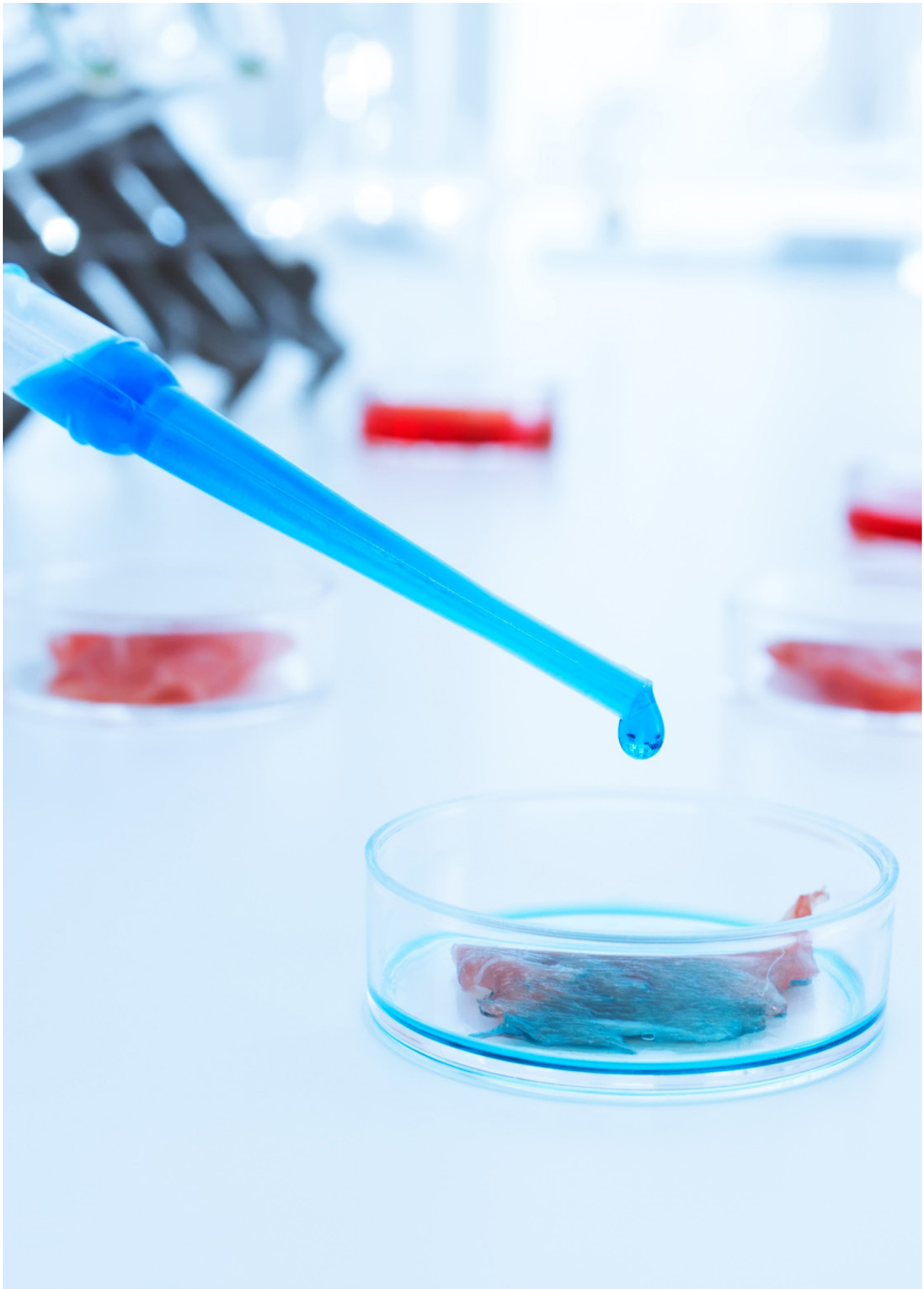
One of ECHA's strategic aims for future years is to become a scientific hub in regulatory science. Together with the European Commission's Joint Research Centre, we promote alternative testing methods and strategies. The priority in the coming years will be on alternative approaches and testing strategies for those endpoints where animal tests would normally be required and especially those which are relevant for the 2018 registration deadline. A large number of registration dossiers are expected for the final deadline and so the positive impact of companies using alternatives to testing on animals would be even greater than before. Furthermore, ECHA is developing a scientific framework to capture the growing experience in the assessment of the validity of read-across cases.

With that in mind, we will use this report's findings to promote alternative methods through publications, our website, guidance documents, and events. We particularly encourage companies registering for the 2018 deadline, to make use of our support.

Even though REACH is European legislation, the work for safer chemicals and alternatives to testing on animals is truly an international cause. We at ECHA are proud to play our part in that. I want to thank companies for the progress made so far and encourage them to develop and make use of alternative testing methods further.

Thank you and I hope that you will find the report of interest.

Geert Dancet
Executive Director



Executive summary

This is the second of the European Chemical Agency's reports on The Use of Alternatives to Testing in Animals, which has been submitted to the European Commission. The first such report from ECHA was published in 2011.

This report is provided to the Commission to fulfil ECHA's obligation under Article 117(3) of the REACH Regulation. It contains the latest information on the status of the implementation and use of non-animal test methods and testing strategies used to generate information on intrinsic properties and for risk assessment to meet the requirements of the REACH Regulation.

WAYS TO AVOID UNNECESSARY TESTING UNDER REACH

It is not the purpose of the quantative analyses conducted for this report to assess the quality of scientific information in registration dossiers, including whether the alternative approaches used comply with the information requirements. The quality of registration dossiers is covered by other activities, such as compliance checks of individual registrations, and by other published information, for example, ECHA's Annual Evaluation Progress Reports and ECHA decisions.

The main findings are as follows:

- The principle in REACH of the sharing and joint submission of data on intrinsic properties of a substance continues to work well. The registrants used it to fulfil the information requirements and to avoid unnecessary animal testing.
- Registrants are making full use of the adaptations outlined in REACH Annexes VII to XI to avoid unnecessary animal testing. The building of categories and predicting substance properties by 'read-across' were commonly used for fulfilling the information requirements, consistent with the findings of the 2011 report. **A 'read-across' or category approach was used in up to 75% of analysed dossiers for at least one endpoint.**
- Registrants started to take up new or revised *in vitro* methods for skin and eye irritation endpoints. The total number of *in vitro* studies submitted for skin and eye irritation has tripled. **Almost 20% of analysed dossiers contained *in vitro* studies for these endpoints.** Registrants have already made use of alternative test methods for the skin sensitisation endpoint, even though this approach is still in its early stage of development.
- Third parties frequently send comments of a scientific nature on testing proposals published on ECHA's website. In a number of cases, registrants appeared to have used the information provided to remove the testing proposals by either submitting an adaptation to cover the information gap or by including experimental data on the substance itself.

ECHA will use the information in this report in its efforts to promote the use of alternative methods through its publications, guidance development, campaigns, events and the Agency's website. The findings of this report will contribute to the development of actions to advise registrants ahead of the 2018 registration deadline.

Under REACH, there are several ways to avoid unnecessary testing. In this section, ECHA highlights three of them: data sharing, the use of read-across and grouping approaches (which have been examined by ECHA) and the third party consultation process conducted as part of the testing proposal examinations.

The sharing of data from tests is a key mechanism for registrants to avoid unnecessary vertebrate animal testing. By the second registration deadline in 2013, ECHA received 8 317 new registrations which were part of joint submissions (the ratio of member to lead dossiers was approximately 3:1). A low percentage (about 0.3%) of member dossiers contained an opt-out for at least one endpoint concerning tests on vertebrate animals. These joint submissions, along with 713 new individual registrations, covered 2 998 phase-in substances at or above 100 tonnes per year. Furthermore, the analyses in this report show that the inquiry process is working and that the majority of successful inquirers submitted their registration as part of a joint submission. ECHA therefore concludes that, in general, data sharing and the joint submission of information is working well.

Read-across has been addressed by ECHA particularly in the context of testing proposal examinations. Registrants made these proposals to generate information which will be used to fill information gaps for groups of substances that they considered to be similar. Some of the larger groups consisted of hydrocarbon solvents, alkenes, petroleum substances, and cobalt-compounds, among others. To support their explanations for their read-across and to justify the selection of the substances within the groups that are to be tested (e.g. to cover structural diversity of the substances), registrants made use of existing information or generated new information (for example, toxicokinetic studies and lower-tier studies). Such testing proposals could be accepted by ECHA and the Member State Committee when the registrants' approach was deemed plausible pending the outcome of the tests. In the event that the tests do not give the predicted outcome, further tests may be needed. ECHA will use the experience gained in these activities to further promote best practice on how to use read-across.

Up to 1 January 2014, ECHA held over 500 public consultations on registrations containing testing proposals submitted by registrants (covering nearly 1 000 tests on vertebrate animals). ECHA received approximately 650 comments, mainly from animal welfare NGOs and, to a lesser extent, industry groups. Relevant experimental studies which address the endpoint and substance under consideration are rarely identified. Most typically, the information provided by third parties raises possibilities for the use of alternative approaches. Registrants receive this information from ECHA. When deciding whether it can be used, either by itself or as an adaptation to fulfil the information requirement, the registrant needs to take into account, for example, whether the information is available for its use (e.g. respecting the data owner's rights) and whether it is of sufficient quality (e.g. whether it contains the necessary experimental details and test material identity). In a number of cases, registrants appeared to have used the information provided by third parties to remove the testing proposals from their registration by either submitting an adaptation to the information gap or by including experimental data on the substance itself.

If the registrant fills the data gap and removes the testing proposal, ECHA terminates the evaluation.

IN-DEPTH ANALYSIS

This section describes the pool of data and methodologies that were used in the data mining analyses. It also addresses the main findings on the use of adaptations, including trends. Furthermore, it summarises findings on new developments regarding skin and eye irritation tests. In addition, the number of new testing proposals and new experimental studies are given.

Methodology

The use of alternatives by registrants was assessed using IT-based data mining techniques to analyse the information that the registrants have submitted to ECHA in their dossiers. The main data pool for the in-depth analyses undertaken for this report are the registrations held by ECHA on 1 October 2013. These mainly comprise registrations of substances imported or manufactured at or greater than 100 tonnes per year. These dossier types (i.e. 100 or more tonnes per year) were selected for in-depth analyses as they are the most data-rich and therefore contain the most information on the use of alternatives by registrants.

The resulting total data pool can be sub-divided into two parts. The first excludes those registration dossiers of categories of substances (compiled by registrants using the respective IUCLID category template) and covered 3 662 substances in 3813 dossiers. This was the data pool used for most of the in-depth analyses conducted for this report. The second part includes the registration dossiers compiled as categories and which covered 523 substances. This was used for analysis of read-across within these dossiers. The total pool of dossiers was used to identify new experimental studies.

This total data pool also contains registrations for substances that were analysed for the first report in this series. As registrations change over time, for example, due to cease of manufacture, tonnage changes, inclusion of new data among others, the snapshot of the information seen in registrations reported in ECHA's 2011 report may no longer reflect the use seen in the same registrations in 2013.

In addition to the analyses performed for the previous report, newly developed data mining techniques have been introduced for this report for more efficient processing but which also allow the generation of new perspectives of the registration data that was not previously possible. One such technique is the unique experimental study (UES) concept used to avoid double counting of unique studies within registration dossiers and allows a more detailed analysis on the use of alternatives to be performed.

Trends identified

Since the previous report, the total pool of registration dossiers (all types) has grown from 24 560 dossiers covering 4 599 substances (on 28 February 2011) to 38 711 dossiers and 8 729 substances (on 1 October 2013). This increase is mainly due to the submission of phase-in registrations for substances imported or manufactured in quantities between 100-1 000 tonnes per year for the second REACH registration deadline of 31 May 2013.

To allow an indication of trends to be identified within the registration dossiers selected for analysis (i.e. 100 or more tonnes per year, excluding IUCLID category dossiers), the percentage change in the types of endpoint study records found have been presented. A particular focus for the discussion of this analysis are the differences between phase-in registrations covering 100 to 1 000 tonnes per year submitted in 2010 compared to those submitted in 2013. The information requirements of Annex IX are common to both of these types of registration. These registration types were selected for greater attention as they provide the most new additional information, which may reflect changes in the approaches taken by registrants in the intervening period. For these registrations, the general overall picture which emerges is that fewer experimental studies were available across all endpoints in the more recent registrations. Consistent with this observation was a greater inclusion of endpoint study records across all endpoints, based on the use of adaptations, especially read-across and weight of evidence. It also appeared that there was a greater use of (Q)SARs for the endpoint bioaccumulation in fish. Given that the standard information requirements are the same for these registrations, these data appear to show that registrants for the 2013 registration deadline appeared to have less existing tests available and made greater use of alternatives such as read-across and weight of evidence.

Registration dossiers (phase-in, 100 or more tonnes per year, excluding IUCLID category dossiers[1]) covering 3 662 substances were also analysed using the "substance approach". This data mining approach gives an insight into the relative proportions of different information types in the registration dossiers. The most frequently used information was from experimental studies and the preferred alternative approach was read-across. It can also be noted that there were markedly fewer experimental studies available for the human health higher-tier endpoints (e.g. sub-chronic, prenatal developmental or reproductive toxicity, required by Annex IX and X of REACH) than for the other endpoints. Weight of evidence was the next most frequently used option. In respect of environmental endpoints, experimental data on short-term toxicity to fish were available for the majority of substances, with read-across being the most frequently used alternative. For bioaccumulation in fish the use of read-across, weight of evidence and experimental data

1 IUCLID dossiers for which registrants used a category template

were used of similar proportions and (Q)SARs were also provided. Generally speaking, these findings are similar to those observed following analyses of the 1 000 tonnes per year registrations in the first report.

Read-across was one of the main adaptations used by registrants. A further analysis was undertaken which showed that read-across or category approach was used for one or more endpoints, which may require tests in vertebrate animals in up to 75% of all of the substances from the total data pool. When analysing dossiers that were not submitted as a category (i.e. without the use of the IUCLID category template), read-across was still found to be used more often than weight of evidence and calculated results/(Q)SARs (equivalent to 72%, 51% and 22% of the substances, respectively). This finding is in line with that from the substance approach, as described above. A further analysis of the dossiers showed that weight of evidence approaches mainly comprised the use of experimental studies (41% overall) and read-across (50% overall). A further analysis showed a calculated result or (Q)SAR was most frequently used for bioaccumulation and to a lesser degree for short and long-term toxicity to fish and skin sensitisation.

Detailed analysis of non-animal test methods for skin and eye irritation

Taking all of the different numerical analyses together, the consistent finding is that use of read-across is the key alternative approach found in the analysed registration dossiers. In particular, this approach is used for the higher tier endpoints where alternative non-animal test methods and testing strategies approved for regulatory use are not yet available.

Endpoints for which non-animal test methods have been recently developed or revised are eye irritation and skin irritation/corrosion. These endpoints were selected for analysis in more detail. Such test methods, if used within the limitations of their applicability domains, can be used to generate information to fulfil the Annex VII information requirements for *in vitro* tests. Furthermore, depending on the test methods used and on the outcomes of these tests, the resulting information may allow adaptation of the information requirements of Annex VIII for the *in vivo* skin and the *in vivo* eye irritation tests by using a weight of evidence approach.

Detailed analysis of the endpoint study records for the skin irritation/corrosion and eye irritation endpoints (all 100 or more tonnes per year registrations excluding category dossiers) showed that registrants mainly used existing data (about 60%) or read-across approaches (about 15%). Almost 20% of the dossiers contained *in vitro* studies, either as the sole information or were provided in combination with other information. New *in vivo* tests alone were observed in 2.5% (skin irritation) or 4% (eye irritation) of the dossiers analysed. The total number of *in vitro* studies submitted for skin and eye irritation endpoints has increased from 442 (in the 2011 report) to 1 410 in 2013. Therefore, it appears that registrants have made use of the available *in vitro* test methods with the aim of fulfilling the standard information requirements of REACH for both *in vitro* and *in vivo* studies. ECHA notes that the approaches taken by registrants appear to follow the currently described practices (OECD and ECHA) for using Integrated Testing Strategy (ITS) approaches.

Testing proposals and new experimental studies

In total, 701 testing proposals on vertebrate animals were submitted in registrations for the 2013 deadline, which ECHA will need to evaluate by 1 June 2016. Of those, 563 were proposals to test on animals in order to fulfil the REACH information requirements listed in Annex IX. The number of proposed tests is slightly less than the 711 testing proposals for vertebrate animal tests made by registrants in 2010. In part, this is due to differences in registration types, which had to be submitted to meet the 2010 and 2013 registration deadlines. The majority of registrations submitted in 2010 concerned the information requirements of Annex X. In contrast, it was mainly 100-1 000 tonne per year registrations (Annex IX information requirements) that were submitted for the 2013 registration deadline. Therefore, the majority of new testing proposals concern the information requirements of Annex IX only. For example, toxicity to reproduction is a standard information requirement in Annex X (231 testing proposals identified from the registrations in 2010) whereas for the registrations submitted by the 2013 deadline

(72 testing proposals), that information only needs to be present if there are identified concerns for this endpoint. The endpoints for which most testing proposals have been made by registrants are for repeated dose toxicity and developmental toxicity studies. This observation appears to be consistent with the endpoint study record analysis conducted for this report, which showed that existing experimental data for these endpoints are less frequently available in 100 to 1 000 tonne per year registrations in 2013 compared to what was seen in the registration dossiers in 2010.

The presentation of 'new' studies (with a "reference date" of 2009 or later) in this report includes all those found in registrations (at 100 or more tonnes per year) held by ECHA on 1 October 2013. Cases were identified using the unique experimental study approach and the analysis now includes new studies found in the category template dossiers and also distinguishes between different registration types. Therefore, the results cannot be directly compared with the findings of the previous report due to the differences in the datamining approaches used. It was found that the cumulative total for the number of "new" experimental studies in these registration types is 7 939 (3 052 *in vitro* and 4 887 *in vivo* experiments).

Many of these new *in vitro* and *in vivo* studies provided by registrants cover the obligatory core information requirements of Annexes VII and VIII of REACH. It can be seen that for those information requirements that are common to both tonnage bands, 100 to 1 000 tonne per year (Annex IX) registrations included a greater number of new *in vitro* tests than for the 1 000 or more tonne per year (Annex X) registration dossiers. In addition, across a number of endpoints, 100-1 000 tonne per year registrations contained more new *in vivo* vertebrate tests than the 1 000 or more tonne per year registrations. The biggest differences (more than two-fold difference) were seen for combined reproductive toxicity screening studies and acute toxicity studies, which is not wholly accounted for by the different numbers of substances covered by these registrations. These observations add to the overall picture of lower availability of already existing experimental studies in the 100-1 000 tonne per year registrations (mainly submitted in 2013) compared to the 1 000 or more tonne per year registrations (mainly submitted in 2010).

To generate quantitative data for the purposes of this report, a computerised search found 293 cases of new higher tier studies in vertebrate animals without a decision from ECHA on a testing proposal or from a compliance check. There can be reasons why new highertier studies are provided in registration dossiers without the need for an ECHA decision (for example, the study was conducted for another regulatory purpose and provided as it was already available). Registrants have previously been advised to provide their reasons for the availability of such studies in their registration dossier but the reasons may not be detectable by computerised searching. From these 293 cases, the registrants indicated that the tests were conducted for other purposes (e.g. non-EU regulatory purposes) in 39 cases and hence a testing proposal has not been necessary. In 73 cases, the registrants were not the data owners and hence may not themselves have commissioned the studies. In another 14 cases, a testing proposal was submitted and when examined by ECHA it was noted that testing was already ongoing or done. In these cases, ECHA has already communicated relevant details to the Member State authorities. In the remaining 167 cases, the computerised search was not able to determine whether the registrant has included an explanation as to why no testing proposal was submitted for these new studies. ECHA has followed the approach for the previously identified similar cases in the 2011 report: the Member State authorities have been informed of the details of these cases for their consideration of any further actions.

SUPPORT FOR REGISTRANTS AND OUTLOOK TOWARDS 2018

Registrations for the 2018 registration deadline must address the standard information requirements of Annex VII and VIII. To achieve this, registrants may need information from vertebrate animal studies to cover the endpoints of irritation/corrosion, skin sensitisation, acute toxicity, repeated dose toxicity, combined reproductive screening assay and mutagenicity. The findings in this report will contribute to deciding ECHA's

priorities to support the development and promotion of non-animal test methods and strategies for those 2018-relevant endpoints.

ECHA supported the introduction of recent advancements in the availability and promotion of *in vitro* test methods for skin and eye irritation. However, there may be substance-specific or classification and labelling-specific limitations within the available *in vitro* test methods. Therefore, the use of the available non-animal test methods in an integrated testing strategy reduces the number of *in vivo* tests that are considered necessary, but some *in vivo* tests may still be needed at this point in time.

Skin sensitisation is one of the endpoints for which most new experimental studies have been conducted according to the analysis in this report. ECHA considers the future implementation of alternative test methods for this endpoint as a priority and is providing support to an OECD-initiated project to develop guidance on the evaluation and application of an Integrated Approaches to Testing and Assessment (IATA). Furthermore, ECHA is collaborating with the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) to translate such an approach into a REACH-specific IATA by 2018.

Acute toxicity is one of the endpoints for which existing and new *in vivo* experimental studies are used most often to address the information requirements. In 2013, EURL ECVAM published a recommendation on the neutral red uptake *in vitro* assay for identifying substances with an oral lethal dose (LD)50>2000 mg/kg, which may have application in a weight of evidence approach in REACH. It is too early to assess if registrants have used this method.

Non-animal test methods to cover the information requirements for repeated dose toxicity studies and combined reproductive toxicity screening studies, whether used individually or in combination, cannot be used to fully replace the corresponding *in vivo* test method. Nor are they considered acceptable for the purposes of fulfilling the respective standard information requirement(s). The use and promotion of read-across, with a focus on repeated dose toxicity, is a priority for ECHA's contribution to the SEURAT-1 project, which aims to integrate the latest scientific developments with current regulatory practice.

An integrated testing strategy that incorporates the adopted *in vitro* mutagenicity test methods is already available. As outlined in the REACH Regulation, registrants must consider the need for appropriate *in vivo* mutagenicity studies if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII. ECHA is consulting stakeholders on a proposed clarification in its guidance that if the registrant considers additional *in vivo* testing to be necessary and such *in vivo* testing falls under Annex IX information requirements, a testing proposal is required even for substances at the Annex VII or VIII level of supply. Non-animal replacements for the *in vivo* mutagenicity tests, which are suitable for regulatory use as specified in the REACH Annexes, are not foreseen to be available by 2018.

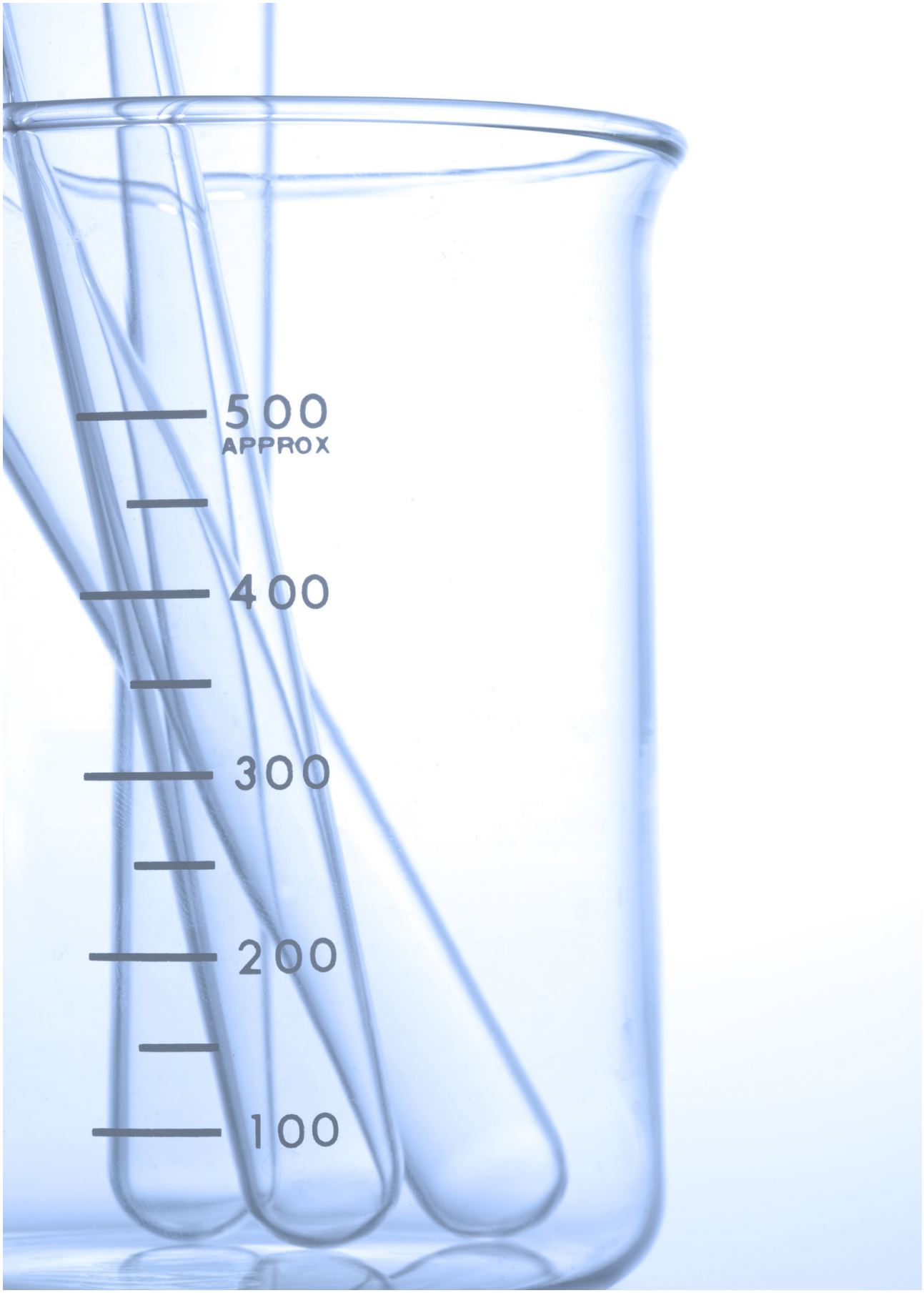
The Fish Embryo Toxicity (FET) Test has been approved as an OECD Test Guideline (OECD 236) in July 2013, and a corresponding EU Test Method will become available in the coming years. It is expected that further information on the use of this new test guideline by registrants will be available for the next report.

ECHA is continuing its efforts to promote the correct use of read-across, and is developing its framework to guide the consistent assessment of read-across and grouping approaches as presented in the registration dossiers. Lessons learnt from this exercise will be used to develop case examples for its website and guidance documents, as well as further advice to registrants and stakeholders.

ECHA continues to support the development of the OECD QSAR Toolbox project with the addition of more functionalities, improved guidance, and training events. Further developments are planned to identify how the programme could accommodate developments in adverse outcome pathways for their use in IATAs.

ECHA supports a number of scientific and regulatory activities e.g. SEURAT and OECD and has formed a number of bilateral agreements with international partners including regulatory bodies in Australia, Canada, Japan and the USA. ECHA is already working with the Joint Research Centre and aims to further develop this cooperation to both influence and benefit from the latest scientific developments. One contribution, for example, will be the use of the findings of this report to identify the development of actions for assisting registrants ahead of the 2018 registration deadline.

This report highlights the use made by registrants of alternatives to testing in animals with the intention of fulfilling the information requirements of REACH. Furthermore, ECHA disseminates the information from registration dossiers and from its decisions on testing proposals and compliance checks. These sources of information will assist registrants and stakeholders to identify the availability of data which may be useful to them, for example, for use in read-across. In addition, ECHA publishes a yearly report on observations and recommendations stemming from dossier and substance evaluations conducted for that year. This accumulated body of evidence will provide all registrants, including those that need to meet the 2018 registration deadline, with the latest information to help them successfully comply with the REACH information requirements.


500
APPROX
400
300
200
100

Preface

This is the second report intended to meet ECHA's legal obligation under Article 117(3) of the REACH Regulation which states that: "*Pursuant to Article 117(3) of the REACH Regulation, every three years the Agency, in accordance with the objective of promoting non-animal test methods, shall submit to the Commission a report on the status of implementation and use of non-animal test methods and testing strategies used to generate information on intrinsic properties and for risk assessment to meet the requirements of this Regulation.*" The first report was published in June 2011.

The primary source of information for these reports is the registration dossiers submitted by manufacturers and importers to ECHA. The results of ECHA's dossier evaluations (compliance checks and examinations of testing proposals) is another source of information but relates only to a fraction of the dossiers submitted.

This report analyses data submitted by the registrants with a view to describing the extent to which alternative test methods and testing strategies have been used. This analysis is complemented by the observations obtained from dossier evaluation. Such findings are also reported by ECHA in its annual evaluation progress reports, which, pursuant to Article 54 of the REACH Regulation, are published in February each year[2].

These reports contribute to the monitoring of the implementation of the REACH Regulation and are intended to provide useful information for the Commission when reviewing the legislation.

2 http://echa.europa.eu/regulations/reach/evaluation

List of abbreviations

AOP	Adverse Outcome Pathway
CASPER IT	Characterisation Application for Selection, Prioritisation, Evaluation and Reporting in relation to registration dossiers
CLP	Classification, Labelling and Packaging Regulation (EC) No 1272/2008
CMR	Carcinogenic, Mutagenic, Reprotoxic substance
Commission	European Commission
DG JRC	Directorate General Joint Research Centre
DPRA	Direct Peptide Reactivity Assay
DSD	Dangerous Substances Directive (67/548/EEC)
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Commercial Chemical Substances
ESR	Endpoint Study Record
EU	European Union
EU-NETVAL	European Union Network of Laboratories for the Validation of Alternative Methods
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
GLP	Good Laboratory Practice
h-CLAT	Human Cell Line Activation Test
HPLC	High Pressure Liquid Chromatography
IATA	Integrated Approaches to Testing and Assessment
ITS	Integrated Testing Strategies
IUCLID	International Uniform ChemicaL Information Database
LR	Lead Registrant

MS	Member State
MSC	Member State Committee
MSCA	Member State Competent Authority
MUSST	Myeloid U397 Skin Sensitisation Test
NONS	Non-phase in substances, which have previously been notified under Directive 67/548EEC and are documented in the European List of Notified Chemical Substances (ELINCS)
OECD	Organisation for Economic Cooperation and Development
PARERE	Preliminary Analysis of REgulatory RElevance
PPORD	Product and Process Oriented Research and Development
(Q)SAR	(Quantitative) Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REACH-IT	Central IT system providing support for REACH processes
SEURAT	Safety Evaluation Ultimately Replacing Animal Testing
SIEF	Substance Information Exchange Forum
TCC	Technical Completeness Check
TG	Test Guideline
TMR	Test Methods Regulation (EC) No 440/2008
tpa	tonnes per year
TSAR	Tracking System for Alternative test methods Review, Validation and Approval in the Context of EU Regulations on Chemicals
UUID	Universally Unique IDentifier (used in software construction)
UES	Unique Experimental Study

List of terms

Alternative approach	Encompasses use of alternative methods, integrated testing strategies (ITS) or integrated approaches to testing and assessment (IATA) to fulfil the standard information requirements specified in REACH.
Alternative (test) method	By contrast to animal test methods; in the context of REACH this mainly relates to the use of *in vitro* methods, (Q)SAR, grouping and read-across (Article 13(1)): "Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, *in vitro* methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)." Alternative test methods can also be *in vivo* tests, but which use fewer animals and/or causes less suffering.
Category	Group of substances with physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity.
Endpoint study record	Record (provided in IUCLID format) of the technical dossier used to report study summaries and robust study summaries of the information derived for the specific endpoint from the original study report. For example, an endpoint study record is produced for an individual experimental study.
Endpoint	Observable or measurable inherent property/data point of a chemical substance. It may refer to a physical-chemical property (such as vapour pressure), or to degradability, or to a biological effect that a given substance has on human health or the environment (e.g. carcinogenicity, irritation, or aquatic toxicity).
Hazard	Property or set of properties of the chemical substance that may cause an adverse health or ecological effect provided there is a sufficient level of exposure.
In chemico **test**	Abiotic assay that measures chemical reactivity e.g. by using High Pressure Liquid Chromatography (HPLC).
In vitro **test**	Literally stands for "in glass" or "in tube". Test taking place outside of the "body" of an organism, usually involving isolated organs, tissues, cells, or biochemical systems.

In vivo **test**	Test conducted within a living organism.
In silico **test**	Test which is done or produced by using computer software or simulation e.g. (Q)SARs.
IUCLID flag	Option used in the IUCLID software to indicate the submitted data type (e.g. experimental data) or their use for regulatory purposes (e.g. confidentiality).
Performance standards	Standards, based on a validated test method, that provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar. Included are: (i) essential test method components; (ii) a minimum list of reference chemicals selected from among the chemicals used to demonstrate the acceptable performance of the validated test method; and (iii) the comparable levels of accuracy and reliability, based on what was obtained for the validated test method, that the proposed test method should demonstrate when evaluated using the minimum list of reference chemicals.
Prediction model	Theoretical formula, algorithm or program used to convert the experimental results obtained by using a test method into a prediction of the property/effect of a given chemical substance.
(Q)SARs and SARs	Theoretical models that can be used to predict in a quantitative or qualitative manner the physicochemical, biological (e.g. (eco) toxicological) and environmental fate properties of compounds from knowledge of their chemical structure. A SAR is a qualitative relationship that relates a (sub)structure to the presence or absence of a property or activity of interest. A (Q)SAR is a mathematical model relating to one or more quantitative parameters, which are derived from the chemical structure, to a quantitative measure of a property or activity.
Read-across	Read-across is an approach for filling data gaps, either by using a category or an analogue approach. For the purposes of the REACH Regulation (Article 13(1)), read-across is considered by ECHA to be an alternative method.
Test (or assay)	Experimental system set up to obtain information on the intrinsic properties or adverse effects of a chemical substance.

List of terms

Test battery	Number of tests taken/used for one specific purpose e.g. several alternative methods, which are required to be performed sequentially to cover one *in vivo* endpoint.
Unique experimental study	Technique used for data mining analyses for the scope of this report to identify a unique study, which is reported more than once in the registration dossier. This technique avoids the multiple counting of the same studies to take into account where a single study is reported more than once in a registration dossier(s). This method is therefore particularly useful in analysis of the category dossiers.
Validated test method	Test method for which the performance characteristics, advantages, and limitations have been adequately determined for a specific purpose.
Validation	Process by which the reliability and relevance of a test method are evaluated for the purpose of supporting a specific use.
Vertebrate animal	Animal that belongs to the subphylum *Vertebrata*, chordates with backbones and spinal columns.

List of legislation

CLP Regulation	Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.
Cosmetics Regulation	Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.
Dangerous Substances Directive	Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances; and its subsequent technical adaptations.
Existing Substances Regulation	Council Regulation (EEC) No 793/93 of 23 March 1993 on the evaluation and control of the risks of existing substances.
Good Laboratory Practice Directive	Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.
Protection of Animals Directive	Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC).
REACH Regulation	Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
Test Methods Regulation	Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

1. Introduction

BACKGROUND

It is a primary objective of the REACH Regulation to ensure a high level of protection of human health and the environment from the hazardous effects of chemicals. The REACH Regulation represents the balance established in the legislative process between the need for generating new information on intrinsic properties of chemical substances using animal tests and the aim of avoiding unnecessary testing. It therefore puts the emphasis on the principle that testing on vertebrate animals shall be undertaken only as a last resort.

A key motivation for developing REACH was to fill information gaps for the large number of substances already in use in the EU, as for many such substances there was inadequate information on their intrinsic properties and the risks that their use may pose. Without a comprehensive set of information on the essential hazardous properties of higher volume chemical substances, registrants cannot undertake a chemical safety assessment and cannot identify appropriate risk management measures to avoid or limit exposure to hazardous substances. In particular, information on properties such as organ toxicity after long-term exposure, the potential to induce cancer, toxicity to the developing foetus, toxicity to the reproductive functions, or long-term aquatic toxicity are often not available for such substances. Some of the studies to assess the properties of substances, specifically for high tonnage registrations, are conducted on experimental animals.

SCOPE

This is the second of the reports produced to meet the Agency's obligation pursuant to Article 117(3) of the REACH Regulation to provide information to the Commission on the status of the implementation and use of non-animal test methods and testing strategies used by registrants. Detailed explanations on how ECHA undertakes this obligation have been provided in the first report[3].

The source of data for the detailed analyses in this report are the registration dossiers held by ECHA in its database as of 1 October 2013. To generate information on the total number of new tests and new testing proposals all registration types were analysed. To examine what information has been submitted by registrants, all registrations for lead and individual dossiers at 100 or more tpa were analysed using computerised data mining techniques. It is these dossiers which contain all the relevant information on tests and adaptation possibilities that have been used by registrants. New

3 http://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2011_en.pdf

for this report is the trend analysis comparing the types of endpoint study records submitted in the registrations in 2010 with those submitted in 2013. In addition, a new fingerprint technique to identify unique experimental studies has been developed which allows a more exhaustive analysis of dossiers containing read-across and/or category approaches to be conducted.

This report also provides registrants, other stakeholders and interested parties with a condensed overview of the options to avoid unnecessary testing. Recent developments in the area of *in vitro* methods have been summarised, and highlights of other alternative approaches outlined in the REACH Regulation (data sharing, read-across and category approach, weight of evidence) are provided. Interested parties may also use the information on ECHA's efforts to support registrants, including continuously updating the database built from the complete dossiers, targeted publications, presented events, and published examples of best practice.

In this report, ECHA provides the status of alternative methods that are already, or soon to be, validated or adopted and also addresses ongoing developments, which may be relevant under REACH. ECHA has previously reminded registrants to be careful when using tools developed in research and development projects or other innovative techniques for predicting properties and data waiving as such tools are not necessarily suitable in the regulatory context to satisfy the requirements of the REACH and CLP regulations. Registrants are advised to be mindful of the limitations of predictions from such techniques, which will depend on the particular method used and may be case-specific. Nevertheless, it may be that non-standard and innovative predictions can serve to build up a fuller picture of the property of a substance as part of a weight of evidence approach, as part of an Integrated Testing Strategy or in Integrated Approaches to Testing and Assessment (IATA), even if the property cannot be predicted adequately for REACH and CLP purposes using the technique alone.

The findings of the report may also be informative, in particular, to small and medium sized companies to help them meet their obligations under REACH. The report aims to ease the companies' efforts with REACH by indicating where financial resources can best be targeted and the latest techniques used to avoid unnecessary animal testing. The report also highlights the data-sharing provisions that require companies registering the same substance to either i) make use of existing studies (contributing to (partial) cost-recovery) or ii) generate new data, by sharing the costs among joint submission members.

As legal and scientific debates are ongoing at the time of drafting, ECHA has excluded from the scope of this report i) an assessment of the use of studies that may in the future be performed according to the OECD Test Guideline 443[4] (EU B.59) for extended one generation reproductive toxicity study (EOGRTS)[5] and ii) the analysis of the use of non-animal test methods and testing strategies in the case of substances falling under the scope of REACH and which are used in cosmetic products, and which also fall under the scope of the Cosmetics Regulation (EC) No 1223/2009[6].

ECHA also reminds the reader that the analyses conducted for this report do not address the qualitative aspects of the content of the information, which is given in the REACH registration dossiers. The quality of dossiers is assessed through the process of compliance check and an overview of these results is published yearly in ECHA's evaluation progress report and reflected in other communication channels.

OUTLINE

The report is divided into a number of sections and appendices as follows:

Following Section 1 outlining the background, scope and structure of the report, Section 2 gives an overview of the legal instruments under REACH designed to make sure that animal testing is only undertaken as a last resort. Section 2 also addresses the obligation to comply with the data sharing and joint submission requirements and the responses

4 http://www.oecd-ilibrary.org/environment/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264185371-en;jsessionid=1ltap7bcp08td.x-oecd-live-02

5 By 1 October 2013, ECHA's database used for data mining did not contain any reproductive toxicity studies performed according to the OECD Test Guideline 443 (EU B.59)

6 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2009R1223:20130711:en:PDF

by third parties to ECHA's consultations on testing proposals. ECHA recommends registrants and the third parties to consider ECHA's assessment of the third party consultation process summarised in Section 2.6.

Section 3 provides summarised analyses obtained from the computerised data mining of registration dossiers. Compared to the time of the first report, the database now includes many more registration dossiers. As with the previous report, different analyses have been conducted to give a more detailed picture of what information was submitted with the intention of covering the REACH requirements. Attention is paid, in particular, to those endpoints for which registrants have obligations to submit information equivalent to that obtained from tests on animals and to those endpoints where new alternative test methods or approaches could have been used. The results of the analyses are presented by endpoint in Appendices 1 to 11. A separate analysis on how the registrants made use of adaptations outlined in Annex XI of the REACH Regulation – read-across and categories approaches, (Q)SARs and weight of evidence options – is addressed in Appendix 12.

In addition, Appendix 13 describes the latest developments impacting (new) test methods and approaches, as alternatives to animal testing, which may be particularly useful for registrants predicting the properties of substances and which may help to fulfil the information requirements of REACH.

Section 4 provides more insight on ECHA's commitment both to promote the use of alternatives and to support registrants. ECHA considers this overview of possible support actions beneficial, in particular, for the companies manufacturing or importing chemical substances from 1 to 100 tonnes per year which are preparing their registration dossiers for the next deadline.

Finally, Section 5 provides the overall summary and conclusions of the report.

2. Legal instruments to avoid unnecessary testing

This section provides an overview of the legal instruments under REACH designed to make sure that (animal) testing is only undertaken as a last resort. It may also serve as a reminder to companies that are already involved in fulfilling their obligations under REACH and to third parties that may contribute to the filling of data gaps by providing scientifically-relevant information through the public consultation process.

Besides the possible adaptations of the standard information requirements (based on the specific conditions listed in column 2 of Annexes VII to X or on the more general conditions given in Annex XI of the regulation), REACH provides the registrant with the possibility to submit information from non-animal tests. Indeed all registrants of the same substance share the same obligation to share any available data, especially related to vertebrate animals, through the data sharing process.

Where no data is available that meets the information requirements on higher-tier tests requiring the use of vertebrate animals, the lead registrant on behalf of all registrants in a joint submission has to submit testing proposals to ECHA and to await its decision before conducting the proposed tests.

2.1 IDENTIFICATION OF THE SUBSTANCE TESTED

ECHA reminds registrants that it is their responsibility to identify and justify the sameness of substances coming from different sources (e.g. manufacturers), as this is the key knowledge and basis for data sharing. Each registrant must confirm the identity of their substance by providing the relevant analytical data in their own registration dossier.

Since the substances subject to REACH need to be registered as manufactured or imported, it can be acceptable to include different compositions of the substance in the same joint submission. This is not to neglect the fact that the presence or absence of an impurity with intrinsic properties may raise the justification for one registrant to opt-out for a specific endpoint from the joint submission. This may also be a reason to require additional testing. Thus, detailed documentation on compositional information of the test substance used is considered as important information to be provided with each endpoint study record in IUCLID, and the test material needs to be representative of the registered substance.

As the impurities may differ according to how the substance is made, the source and manufacturing process are essential information for the identification of UVCB substances.

From the assessment performed on the registration dossiers, ECHA identified one typical shortcoming: the failure to identify structural isomers of all kinds, which may have different toxicological properties. The use of screening and predictive tools may be useful to capture these potential differences, if there is no available information on the impurities as individual substances.

2.2 DATA SHARING AND JOINT SUBMISSION AS TOOLS FOR AVOIDING UNNECESSARY TESTING

The obligation to share data applies to any registrant under the REACH Regulation irrespective of the phase-in or non-phase-in status of their substance.

More specifically, pursuant to Articles 27 and 30, potential registrants have an obligation to request that studies involving vertebrate animals are shared and may share studies not involving vertebrate animals. Furthermore, a study involving vertebrate animals that cannot be obtained from another registrant or potential registrant of the same substance, can be conducted by a registrant, unless REACH stipulates that a testing proposal must first be submitted which must be approved by ECHA. Consequently, potential registrants of the same substance are required to collaborate to share the requested information and agree on the data to be submitted jointly.

In accordance with Articles 11 and 19 of REACH, when a substance is intended to be registered by more than one legal entity, the information on the classification and labelling of the substance, study summaries, robust study summaries and testing proposals are to be submitted by one registrant (the lead registrant) acting on behalf of, and with the agreement of the other assenting registrants. That is to say, a joint submission shall be created.

Since the publication of the first report, ECHA has further supported registrants by developing a new IT feature,[7] which facilitates the sharing of registrants' contact details and information about their respective roles in the joint submission. This helps the registrants to continue sharing data, also after registration. It is important to re-emphasise that the REACH Regulation is clear on the concept of joint submission and that it provides for a few scenarios where it is acceptable to submit a registration dossier outside of a joint submission (Article 11(3)). For example, registrants may choose to register individually if submitting the information jointly would lead to disproportional costs, or it would lead to disclosure of confidential information. The registrants may also disagree on the selection of the information to be submitted jointly.

Registrants within a joint submission who need to update their dossier because their reported tonnage increased, also have to comply with additional testing requirements. These registrants have to inquire, pursuant to Article 12(2), whether the data they require have already been submitted to ECHA.

2.3 ACCESS TO DATA SUBMITTED MORE THAN 12 YEARS PREVIOUSLY

To further promote the sharing of data and limit the duplication of animal testing, while protecting the assets of data owners, Article 25(3) of the REACH Regulation provides that any (robust) study summary submitted 12 years previously, to the Agency or any national competent authority (accounting for the previous legislations) in the framework of a registration as defined under REACH, can be used for the purposes of a registration by another manufacturer or importer. ECHA's Guidance on data sharing[8] further indicates that the data can be used for the purpose of a REACH registration without compensating the data owner.

Therefore, ECHA is the "repository" authority of this wealth of information. It is important to note that data that have been generated more than 12 years previously but were never submitted to a competent authority are not subject to the provision of Article 25(3).

As part of the inquiry process, where ECHA identifies that a previous registrant has submitted data more than 12 years previously, it systematically provides an extract of the IUCLID registration corresponding

7 http://www.echa.europa.eu/documents/10162/6340370/na_12_46_reach-itusers_en.pdf

8 http://echa.europa.eu/guidance-documents/guidance-on-reach

to the information requirements of the inquirer. This process was implemented to directly reflect the provision of Article 25(3). However, ECHA is considering amending its process.

In parallel, ECHA has set up a procedure similar to the inquiry process, to address any request for data, which falls under the scope of Article 25(3). To benefit from this procedure, requesters have to demonstrate that they intend to "use [the requested data] for the purposes of a registration" according to the REACH Regulation.

2.4 DATA HOLDERS

Article 28(7) of the REACH Regulation enables manufacturers and importers of phase-in substances, in quantities less than one tonne per year, as well as downstream users of those substances and third parties holding information on those substances, to submit information to ECHA and consequently to become part of the SIEF. The information submitted by those "data holders" has been handled automatically through REACH-IT in such a way that pre-SIEF participants can identify the holders of data that can help to fill the possible data gaps. Pre-SIEF participants have been reminded to contact data holders and request data and cost sharing, where relevant.

ECHA has had no involvement regarding whether pre-SIEF participants make use of this opportunity to limit the duplication of animal testing.

2.5 TESTING PROPOSALS: OUTCOME FROM THE FIRST REGISTRATION DEADLINE

Pursuant to Article 10(a)(ix) of the REACH Regulation, should registrants identify a data gap, they should not undertake any new Annex IX or Annex X studies before submitting a testing proposal to ECHA and receiving ECHA's decision. A detailed explanation on the testing proposal examination process is provided on ECHA's website and in ECHA's annual evaluation progress reports.

For the testing proposals for phase-in substances submitted by the first registration deadline of 30 November 2010, ECHA had to prepare a draft decision by 1 December 2012[9]. A total of 571 dossiers (with altogether 1 184 individual testing proposals from which 711 were tests on vertebrate animals) were examined and the deadline has been met successfully. Detailed information on this outcome can be found in the evaluation progress reports of 2012 and 2013[10].

ECHA has noted that in many cases the registrants applied a read-across approach. For example, when analysing data for the first Article 117(3) report, ECHA noted 711 proposed tests which would require vertebrate animals. Among them, ECHA identified that they included 78 substances that were submitted as category dossiers, covering 17 chemical substance categories and testing proposals for 104 animal studies (cut-off analysis date: 28 February 2011). This indicates that registrants made an effort to cover the identified data gaps by applying the abovementioned approaches.

As outlined above, all testing proposals submitted before 30 November 2010 have been examined by ECHA and draft decisions have been sent to the registrants. In 2013 and 2014, evaluation continued. Especially when concluding on category and read-across approach, ECHA put substantial effort in to communicating the noted shortcomings of testing plans, hypotheses and proper justifications to the registrants. The registrants did improve the quality of their dossiers.

Taking into account substantial efforts from both ECHA and industry, it is noteworthy that during the discussions on testing proposal examination under REACH at the Member State Committee (MSC) meetings (in 2013 and 2014), the Committee agreed on a read-across approach for 12 cobalt salts, 22 petroleum substances, 27 alkenes and 35 hydrocarbons[11], as proposed by the registrants after improvement of their dossiers.

9 http://echa.europa.eu/view-article/-/journal_content/title/first-wave-of-proposals-to-test-substances-examined

10 http://echa.europa.eu/regulations/reach/evaluation

11 http://echa.europa.eu/about-us/who-we-are/member-state-committee/meetings-of-the-member-state-committees

Table 1: Overview of testing proposal categories addressed by ECHA and the MSC by March 2014

Category	Number of substances	Number of test substances	Endpoints	Number of test(s)
Cobalts	12	2	Sub-chronic toxicity study	2
			Pre-natal developmental toxicity study	2
			Two-generation reproductive toxicity study	2[12]
Alkenes	27	5	Sub-chronic toxicity study	5
			Pre-natal developmental toxicity study	5
			Two-generation reproductive toxicity study	5
Petroleum substances	22	6	Pre-natal developmental toxicity study	2
			Two-generation reproductive toxicity study	6
Hydrocarbon solvents	35	3	Two-generation reproductive toxicity study	3
In total	96	16		32

Regarding petroleum substances, the MSC agreed with ECHA not to accept the category approach, but to accept the testing plan proposed as plausible based on the one-to-one read-across. In all other cases, the MSC agreed to the category and the read-across approach proposed by the registrants and reflected in ECHA's draft decisions recognising the uncertainties still present in the approach and stating that although the hypothesis may be tested, ECHA will consider acceptability of the read-across only when the information required by the decision has been submitted to ECHA and the Agency has evaluated the information.

From the 12 cobalt salts, two substances will be tested. For the petroleum substances, six of the 22 substances will be tested, followed by one-to-one read-across, while five of the 27 alkenes and three of 35 hydrocarbon substances will be tested. These examples illustrate the considerable saving potential of the category and read-across approach, when it is applied in an appropriate manner. The overview of those categories is provided in Table 1.

12 All 16 listed decisions on two-generation reproductive study endpoint pending with the European Commission.

In all cases, if the proposed tests would not confirm the read-across hypothesis relied upon by the registrants, this shall not alter the obligation of the registrants to meet the standard information requirements. Should the read-across strategy be inadequate, it remains the responsibility of the registrant to ultimately submit reliable information or adaptations, which should not underestimate the hazards of the registered substances in relation to the relevant endpoints. If the proposed approach does not satisfy the conditions set out in Annex XI, ECHA reserves the right to request the information necessary to fulfil the information requirements for the substances as mentioned above.

2.6 THIRD PARTY CONSULTATIONS FOR TESTING PROPOSAL EXAMINATIONS

A purpose of the consultation is to provide an opportunity for submission of any valid information or data that addresses the relevant substance and hazard endpoint addressed by the testing proposal. This is one of the ways to make sure that tests on

vertebrate animals conducted for the purposes of the REACH Regulation are only performed if necessary. Such information, if it can be used in the fulfilling of the data gap, may mean that the proposed testing is no longer required.

The third party consultations concerning the testing proposals for vertebrate animal tests found in registrations submitted for the first registration deadline (1 December 2010) have been completed. These consultations concern the information requirements of Annexes IX/X (for example, pre-natal developmental toxicity study or long-term toxicity to fish). Valid testing proposals made for the registrations submitted for the 2013 registration deadline are already subject to consultation – the first consultations have been launched.

Up to 1 January 2014, ECHA held 548 public consultations on testing proposals covering 978 tests. ECHA received 650 comments during these consultations. ECHA transmits these comments to the registrant at the time that a draft decision is sent.

Comments are most often provided by international non-governmental organisations concerned with animal welfare (66%) or companies/trade associations with interests in the substance (26%). Typically, the comments are scientific in nature and concerned with addressing the substance and endpoint, which is the subject of the testing proposal. Occasionally, information about the availability of tests for the endpoint and substance concerned is provided. For example, an NGO and a company highlighted the existence of a study outside the EU. The registrant considered the information, negotiated access with the dataowner, incorporated the information into their registration and withdrew the testing proposal.

In the annual evaluation progress reports, ECHA noted that many third party comments are concerned with potential strategies (e.g. citing the possible availability of information, which may support weight of evidence approaches and/or read-across). There are a number of factors, which will need to be taken into account by the registrants if these strategies and information is to be converted into adaptations that fulfil the information requirements so that new testing is not needed. For example, registrants have the possibility to adapt the standard testing regime if information is available, relevant and scientifically valid. These rules may require that reliable and adequate documentation is provided, that the information is useful for the purposes of classification and/or risk assessment or has adequate and reliable coverage of the key parameters addressed in the corresponding test method.

Approaches proposed by third parties may also have referred to the open literature to identify potentially relevant study reports or publications. Registrants may need to acquire and check such reports to make sure that the critical details (experimental details, substance identity) are present to enable its use for fulfilling an information requirement.

One potential hindrance for third parties and registrants is access to information from study reports identified in third party comments. For example, to obtain a report there may be a need to agree access to the data, remuneration of the data holder, agreeing an approach within a SIEF, the need to generate (robust) study summaries of the data and also include them in the update of the registration dossier.

Analysis of the impact of third party comments on registration using IT-tools is challenging, because registrants are not obliged to record their considerations on any third party information they receive. As such, it is unclear whether the receipt and details of third party comments motivated the registrants to adapt their strategies or whether the approach had already been considered and rejected.

In the previous annual evaluation progress reports, ECHA provided examples of third party comments that were followed by a change in testing strategy of the registrant.

Not all third party comments received so far are reflected in published decisions. For example, consultations concerning the information requirement for a two-generation reproductive toxicity study attracted third party comments advocating the use of the extended one-generation reproductive toxicity study. As unanimous agreement by the MSC on the possible use of this method cannot be reached, all draft decisions containing a requirement for a two-generation reproductive toxicity study have been referred to

the Commission for decision making. Registrants will also have a considerable period of time to consider any other information that third parties have provided until there is a final decision.

2.7 HOW DID REGISTRANTS USE THE LEGAL INSTRUMENTS TO FULFIL THEIR OBLIGATIONS?

The analysis of the registration dossiers provides statistical insights on how the registrants have met their obligations to share data. In line with the findings outlined in the first ECHA report, the joint submission of information generally worked well as shown by the high proportion of registrations submitted jointly in relation to phase-in substances subject to the second registration deadline foreseen under REACH[13].

By the second registration deadline, ECHA received 8 317 new registrations from joint submissions (2 156 lead and 6 161 member dossiers) and 713 new individual registrations, covering 2 998 phase-in substances. Most of the phase-in substances were registered by groups of companies working together in joint submissions (83%). The joint submissions have one lead registrant and, on average, 2.9 members. ECHA notes that this ratio average was 6.9 for the registrations submitted by the first deadline. This could be explained by the fact that many commodity substances were registered by the end of November 2010.

When analysing dossiers for the purpose of the first report, ECHA noted that from nearly 3 000 joints submissions (at or above 1 000 tpa), covering almost 20 000 registrations, only 135 member dossiers (less than 0.7%) included an opt-out for one or more endpoints. For the analysis of this report, ECHA screened the updated database containing more than 26 000 registration dossiers (at or above 100 tpa; see Section 3.1 for detailed information). From those dossiers, covering almost 5 400 substances, less than 1% of member dossiers contained opt-outs for at least one endpoint. The reasons provided were mostly based on Article 11(3)(c) (namely "disagreement on the selection of information submitted by the lead registrant").

More specifically, the member registrants provided additional information to fulfil requirements that the lead dossier did not cover due to different tonnage bands and registration types (full registration and intermediates) within the joint submission. A low rate (about 0.3%) of those endpoint-specific opt-outs were directly referring to the endpoints, which require vertebrate animal testing.

By March 2014, ECHA together with the Member State Committee have rejected four testing proposals submitted by the registrants. This indicates that in general, registrants made use of the available data and adaptations of information requirements before asking for new tests.

Between 2008 and 2013, ECHA received more than 8 000 inquiries, of which 6 000 were successfully submitted by inquirers. Approximately 1 800 of these inquiries contained requests for data which ECHA can correlate to fulfil the obligation of sharing data with existing registrants. Subsequently, approximately 1 200 potential registrants registered their substance, benefiting from information provided during the inquiry process. ECHA considered this to be a measurable contribution to the limitation of duplication of testing. ECHA noticed that the numbers of such requests for information submitted within the inquiry process have followed the same trend over the years, with a peak of requests one year ahead of each upcoming deadline. By the end of 2013, ECHA processed more than 300 requests to access data submitted more than 12 years previously. ECHA also reminded that it is the registrant's responsibility to make sure that the data received is properly used and assessed.

Following the inquiry process, registrants of (not pre-registered) phase-in substances complied better with their joint submission obligations (88%), in comparison with registrants of non-phase-in substances (40%). ECHA also noted two other elements: 20% of potential registrants never proceeded with registration, although they successfully inquired (including receiving information from ECHA on available data) and on the other hand, approximately 25% of inquirers that did not succeed in submitting their inquiry went on and registered their substance (most of them had not made any request for testing information).

13 http://echa.europa.eu/information-on-chemicals/registered-substances/reach-2013/substances

Overall, following the inquiry process, the majority of successful inquirers submitted their registration as part of a joint submission, fulfilling both their data sharing and joint submission obligations, and thus avoiding the duplication of (animal) tests.

ECHA therefore concludes that, in general, data sharing and the joint submission of information worked well and registrants used them to fulfil their information requirements efficiently. This conclusion is also in line with the previous findings reported in 2011. In addition, ECHA concludes that, in general, registrants also made use of already available information and possible adaptations and did not rush to propose new higher tier vertebrate animal tests.

3. Statistical analysis of endpoint data in the registration dossiers submitted

This section outlines ECHA's analysis performed on available registration dossiers falling under the scope of this report.

As far as it is possible, to keep a level of consistency and comparability between the first and the second Article 117(3) reports, a similar approach to data analysis and data presentation was followed. Therefore, similar dossier selection criteria and algorithms were used to define the initial pool of dossiers subject to analysis. The whole of the current database of dossiers was first screened and those dossiers that fulfilled the selection criteria were further analysed.

3.1 METHODOLOGY

DOSSIER SELECTION AND SCOPE OF ANALYSIS

Annexes VII, VIII, IX and X of the REACH specify what information is required at levels of 1, 10, 100, or 1 000 or more tonnes per year per registrant, respectively. These are called the 'standard information requirements' and are highest for substances produced at or above 1 000 tonnes per year. Therefore, as for the first report, the available most data rich dossiers – standard registration dossiers falling under Annex IX and X information requirements – have been selected for data mining.

The data pool of dossiers for detailed analysis (see below for exclusions) were those submitted by the first and second registration deadlines (30 November 2010 and 31 May 2013), phase-in and non-phase-in substances at or above 100 tpa and at or above 1 000 tpa. Dossiers may have been updated by registrants after submission and so, for this analysis, the data that were available up to a cut-off date of 1 October 2013 were used.

ECHA notes that its dossiers database is constantly changing (dossiers being updated, testing proposals withdrawn, companies ceasing manufacture of their substances and changing their role under REACH in the SIEF). For the purposes of this report, ECHA did not perform any separate analysis targeted on updates of the 2010 registration dossiers. Neither did ECHA examine the reasons for the late registrations (dossiers submitted after the legal deadline, depending on the tonnage band). ECHA stresses that it is the responsibility of the registrants to submit their dossiers in due time.

In line with the previous analysis performed in 2011, certain submissions were again excluded from the reports scope. These included substances manufactured or imported only for use as intermediates under strictly controlled conditions, substances notified for use in product and process-orientated research and development (so-called

'PPORD's') and notified substances under the former regulatory scheme (so-called 'NONS' substances) for which no update in respect of a tonnage band increase had been received. A detailed explanation on why such registrations do not fall under the scope of the analysis has already been provided in the first report pursuant to Article 117(3).

As already explained in the previous report, endpoint study records are specific entries filled by registrants for the hazard endpoints in the IUCLID dossiers. There may be more than one or even many endpoint study records submitted per endpoint and per registered substance. The type of endpoints requested for each tonnage band is specified in Annexes VII-X of the REACH Regulation.

In Sections 3.2 to 3.6, the statistical analysis of endpoint information from four perspectives is described, consistently with the analysis performed for the first report in 2011:

1) from the **endpoint study record** perspective (further called in this report "**ESR approach**"), which analyses the overall quantitative picture of options used by registrants for dossiers within the scope of this analysis (see Section 3.2, Tables 3 to 5, and Appendices 1 to 11)

2) from the **substance** perspective further called "**substance approach**". This perspective focuses on the strategic choices the registrants have made to fulfil the information requirements (see Section 3.3, Figures 2 and 3 and Appendices 2 & 3)

3) from the **combined** perspective where alternative methods have been reported within a weight of evidence approach. This information in missing in two main types of analysis (ESR approach and substance approach). Therefore more detailed analysis of non-test alternatives used by registrants to fulfil REACH data requirements, such as: (Q)SARs read-across and categories and weight of evidence has been performed (see Appendix 12). More specifically, Sections 12.1.1 and 12.2 of Appendix 12 contain quantitative analysis of ESRs where use of read-across and (Q)SARs and their projection versus the context in which these alternatives have been used by registrants (key study, supporting study, weight of evidence) was reported in non category dossiers, and

4) from the perspective of vertebrate animal testing for REACH purposes to find out how many new studies have been performed for REACH or are planned to be performed for REACH, including proposed tests (see Sections 3.5 and 3.6).

It is worth mentioning that in the case of the substance approach, the methodology applied remained the same as that used in the first report. However, this time the scope of analysis has been extended to also cover non-phase-in substances and Annex IX substances as many more of these are now available. As in the previous report, only individual submissions and lead dossiers from joint submissions have been analysed while category dossiers were excluded. It is also noteworthy that for the first time a more in-depth analysis on use of read-across approaches, categories, (Q)SARs and weight of evidence has been performed (see Section 3.4 and Appendix 12).

The in-depth analysis of registration data for this report is on registrations received and successfully processed by 1 October 2013 for both phase-in and non-phase-in substances at or above 100 tonnes per year. These dossiers should contain the core data of Annex VII, VIII and IX in order to be accepted for registration (and pass the technical completeness check). If data gaps have been identified by the registrants, that could not be filled otherwise, where appropriate, they should contain testing proposals for the necessary higher-tier studies of Annex IX. In addition, registration dossiers for both phase-in and non-phase-in substances at or above 1 000 tonnes per year and their updates were analysed.

As shown in Table 2, by the 1 October 2013, the total number of registration dossiers available for the analysis was 38 711 (i.e. 8 729 substances). Following the second registration deadline for phase-in substances of 31 May 2013, ECHA received 9 030 new registration dossiers from which 2 998 were phase-in substances manufactured or imported in quantities of 100 tonnes or more per year. Additional substances consisted of registrations covering non-phase-in substances and (updates of) dossiers for substances registered by the 30 November 2010.

From the original number of 38 711 registration dossiers, 26 171 dossiers were identified to be

Table 2: Registration dossiers within the scope of in-depth analysis of this report

Tonnage band	Phase-in	Non phase-in	Total
All	35 082	3 629	38 711 (8 729 substances)
Registration dossiers with tonnage band > 100 tpa			
> 1 000 tpa	17 080	569	26 171 (4 118 substances)
100 - <1 000 tpa	8 522		
Registration dossiers excluding category dossiers			
> 1 000 tpa	16 545	562	25 522 (4 004 substances)
100 - <1 000 tpa	8 415		
Lead and individual dossiers			
> 1 000 tpa	1 742	364	3 988 (3 788 substances)
100 - <1 000 tpa	1 882		
Lead and individual dossiers (excluding NONs without tonnage upgrade)			
> 1 000 tpa	1 740	203	3 813 (3 662 substances)
100 - <1 000 tpa	1 870		

registration dossiers with a tonnage band at or above 100 tonnes per year (including those at or above 1 000 tpa). As done previously, it was necessary to exclude dossiers for 'chemical categories' from the in-depth analysis, as those dossiers were analysed separately. At or above 100 tonnes per year, there were 649 (533 lead and individual dossiers) IUCLID category dossiers covering 121 categories and 523 substances (some of those substances also being covered by standard registration dossiers). This exclusion further diminished a number of analysed dossiers to 25 552.

From the remaining 25 552 dossiers, only the lead registrant's dossiers, dossiers submitted that used the opt-out provisions for specific endpoints and dossiers for individual registrations contained endpoint information for the registered substances. As mentioned, NONs for which no tonnage upgrade has been received, dossiers for intermediates used under strictly controlled conditions, PPORD notifications and substances with tonnages below 100 tonnes per year were out of the scope of analysis, as only lead and individual dossiers for substances registered at or above 100 tonnes per year contain most of the data on performed animal studies. Thus, the total number of dossiers to be considered for the in-depth analyses was reduced to 3 813, and the number of substances covered reduced to 3 662.

These dossiers were analysed within three groups: dossiers for phase-in substance at or above 1 000 tonnes per year, phase-in substance dossiers between 100 and 1 000 tonnes per year and non-phase-in dossiers with a tonnage band of 100 tonnes per year or more.

To analyse data submitted by registrants in the registration procedure to ECHA, the Agency has developed an IT application allowing identification of substances fulfilling pre-defined criteria. These selection criteria were designed to find and extract information on the number and type of different options used by the registrant to meet the information requirements under REACH.

Furthermore, an additional and more detailed examination of the registration information available for the skin corrosion/irritation, eye irritation and skin sensitisation endpoints was performed as new and revised test guidelines have become available since the data was analysed for the first report in this series.

In line with the analyses performed in the first report, an estimation of the number of vertebrate animals saved through the use of alternative approaches in REACH was not conducted, due to the significant number of (debatable) assumptions required to perform such an analysis.

Further information on the selection criteria applied in data extraction and analysis for the individual endpoints is presented in Figure 1.

The more detailed analysis on read-across and categories required the possibility to remove duplicate endpoint study records from the analysis, and has thus led to the development and implementation of the unique experimental study (UES) concept. The UES concept was introduced because the same ESR that is used in read-across or category approaches may be used in several dossiers, hence by using a unique identifier it is possible to count a specific ESR only once and thereby avoiding double counting.

COMPARISON TO THE FINDINGS OF THE PREVIOUS REPORT

Due to the more advanced extraction methodology, which has been applied in the current report and different source of input data (i.e. data were directly extracted from the selected pool of dossiers in IUCLID and REACH-IT), ECHA strongly recommends not performing a direct comparison between the findings of the first and the second reports pursuant to Article 117(3) of REACH. The differences in the input data sources may lead to potential data inconsistencies and consequently may impact and/or bias the results: the data pool used for current analysis covers more than double the number of dossiers and substances if compared with the first report. ECHA, therefore, only performed a limited comparison of general numbers and noted similar tendencies.

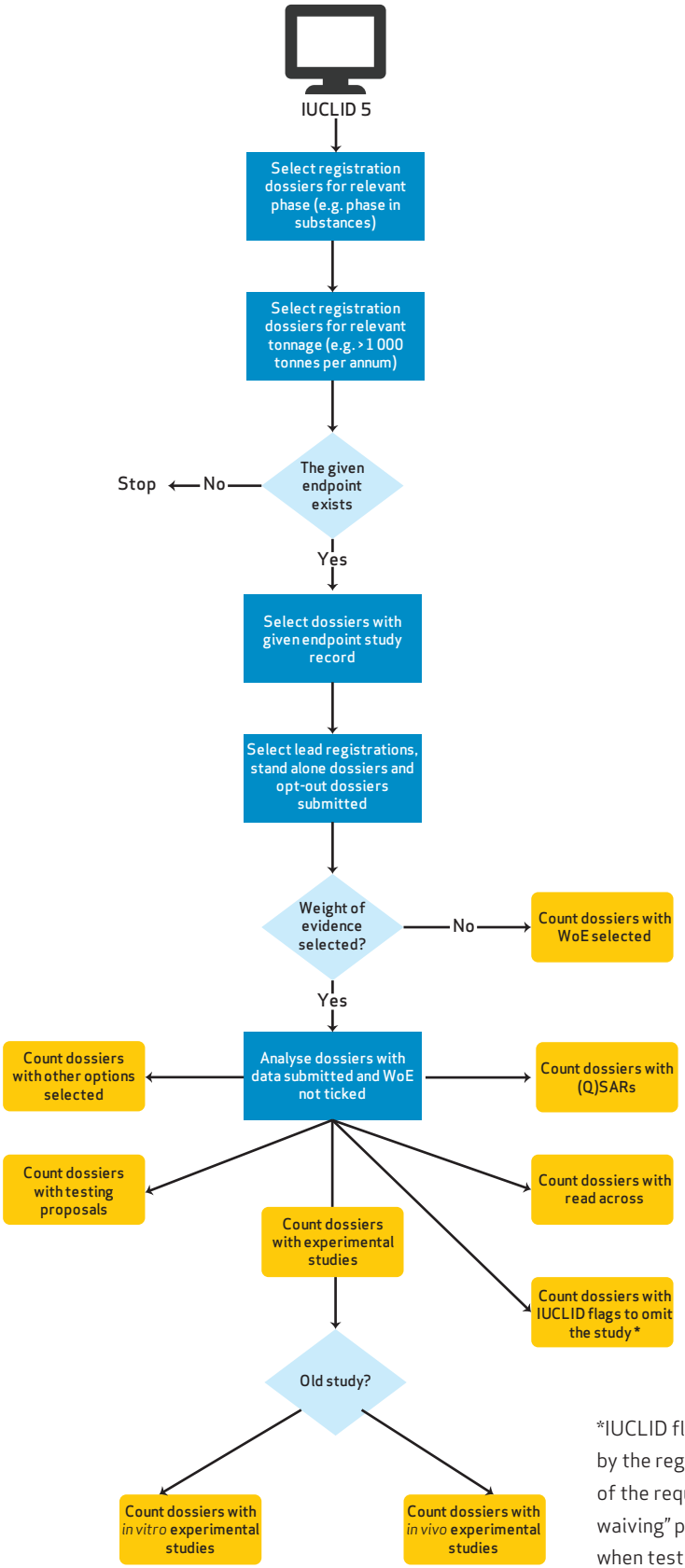


Figure 1: Schematic of data analysis for individual endpoints

*IUCLID flags to omit the study are set by the registrant to omit the submission of the required data filling the "data waiving" pick-list. These are used when testing does not appear to be: scientifically necessary; technically not possible; or not necessarily based on low exposure considerations.

3.2 ESR APPROACH

The ESR approach consists of the analysis of all endpoint study records submitted for the 3 813 dossiers for a given endpoint. For each endpoint, more than one or even many endpoint study records may be available and were summarised for these dossiers (see below). The ESR approach provides the overall quantitative picture of options used by registrants for dossiers within the scope of this analysis.

The results of this approach show what information has been submitted for a given endpoint cumulatively in all dossiers. This analysis provides an overall data availability for endpoints. However, it does not cover which of these data have been used as key data to fulfil the information requirements and it does not allow the degree to which data redundancy is involved per substance to be assessed.

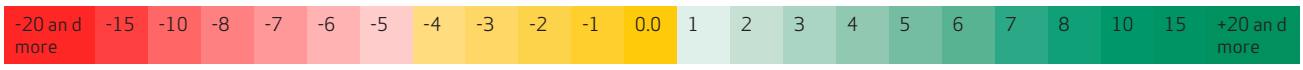
Considering that there were no major changes in the standard information requirements under REACH, ECHA did not expect major changes in the registrants' behaviour except for certain endpoints. These are, as described above, skin and eye irritation, skin sensitisation and bioaccumulation (in the case of vertebrate animal testing) endpoints, for which new test guidelines or other alternative methods have become available. These endpoints were therefore subjects for a more exhaustive analysis.

Table 3 to Table 5 present the available data from an endpoint study record (ESR) perspective in detail. It shows the number of ESRs available in the registration dossiers to fulfil a specific endpoint as reported in the first report or submitted by 1 October 2013 for phase-in or non-phase-in substances in a given tonnage band. During the creation of study records in IUCLID, the registrant can classify them according to the purpose of that study record. The 'Total ESR' shows the total number of study records in the IUCLID dossiers. The rest of the columns contain the number of study records according to the classification assigned by the registrant:

- Column 5 "Experimental studies" contains the number of ESRs classified as "experimental result" from the pick-list in the field "Study result type" (abbreviation: ES)
- Column 6 "Testing proposal" shows the number of ESRs employed by the registrant for the testing proposals. These are classified by the registrant as "experimental study planned" from the pick-list of options in the field "Study result type" (abbreviation TP).
- Column 7 "Read-across" contains the number of ESRs classified by the registrant as read-across from the pick-list of options in the field "Study result type" (abbreviation RA).
- Column 8 "IUCLID flags to omit the study": selected by the registrant to omit the submission of the required data by choosing the appropriate option from those available in the pick-list in the field called "data waiving". These options are to be used to indicate when testing does not appear to be, for example, scientifically necessary; technically not possible; or not necessary based on low exposure considerations (abbreviation FO).
- Column 9 "Weight of Evidence" consists of the number of ESRs classified by the registrant as weight of evidence in the "purpose flag" pick-list. All cases selected as weight of evidence, were counted and not taken into account in more detailed analysis (abbreviation WE).
- Column 10 "(Q)SAR studies" has the number of ESRs classified by the registrant as "(Q)SAR studies" from the pick-list of options in the field "Study result type" (abbreviation QS).
- Column 11 "Miscellaneous" shows the number of ESRs classified by the registrant as "other" from the pick-list of options in the field "Study result type" (abbreviation MS).

Table 3: Human Health Endpoint Study Records: Dossiers subject to analysis in 2011 and 2014 (cut off date 1 October 2013)

1. Test type		2. Phase	3. Tonnage band	4. Total ESR	5. Experimental studis (ES)	%	6. Testing proposals (TP)	%
1. Acute toxicity (all routes)	2011	Phase-in	>1 000	12 874	7 328	56.9	0	0.0
		Phase-in	100 to 1 000	1 649	988	59.9	0	0.0
		Non-Phase-In	>100	396	154	38.9	0	0.0
	2014	Phase-in	>1 000	15 987	9 355	58.5	0	0.0
		Phase-in	100 to 1 000	10 854	4625	42.6	0	0.0
		Non-Phase-In	>100	890	446	50.1	0	0.0
	Difference	Phase-in	>1 000	3 113	2 027	1.6	0	0.0
		Phase-in	100 to 1 000	9 205	3 637	-17.3	0	0
		Non-Phase-In	>100	494	292	11.2	0	0.0
2.1 Skin irritation (*in vitro*)	2011	Phase-in	>1 000	329	252	76.6	0	0.0
		Phase-in	100 to 1 000	24	20	83.3	0	0.0
		Non-Phase-In	>100	1	1	100	0	0.0
	2014	Phase-in	>1 000	443	339	76.5	0	0.0
		Phase-in	100 to 1 000	684	479	70	0	0
		Non-Phase-In	>100	35	29	82.9	0	0
	Difference	Phase-in	>1 000	114	87	-0.1	0	0.0
		Phase-in	100 to 1 000	660	459	-13.3	0	0.0
		Non-Phase-In	>100	34	28	-17.1	0	0.0
2.2 Skin irritation (*in vivo*)	2011	Phase-in	>1 000	5 216	3 343	64.1	0	0.0
		Phase-in	100 to 1 000	600	402	67	0	0.0
		Non-Phase-In	>1 000	157	72	45.9	0	0.0
	2014	Phase-in	>1 000	6 676	4 482	67.1	0	0.0
		Phase-in	100 to 1 000	4 198	2 035	48.5	0	0.0
		Non-Phase-In	>1 000	347	208	59.9	0	0.0
	Difference	Phase-in	>1 000	1 460	1 139	3	0	0.0
		Phase-in	100 to 1 000	3 598	1 633	-18.5	0	0.0
		Non-Phase-In	>1 000	190	136	14.1	0	0.0
3.1 Eye irritation (*in vitro*)	2011	Phase-in	>1 000	172	149	86.6	0	0.0
		Phase-in	100 to 1 000	27	19	70.4	0	0.0
		Non-Phase-In	>100	1	1	100	0	0.0
	2014	Phase-in	>1 000	250	195	78	0	0.0
		Phase-in	100 to 1 000	500	348	69.6	0	0.0
		Non-Phase-In	>100	28	21	75	0	0.0
	Difference	Phase-in	>1 000	78	46	-8.6	0	0.0
		Phase-in	100 to 1 000	473	329	-0.8	0	0.0
		Non-Phase-In	>100	27	20	-25.0	0	0.0



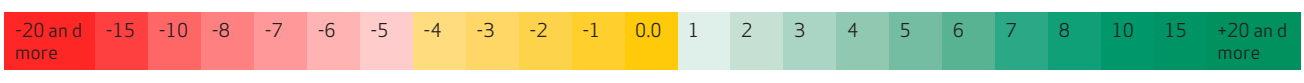
7. Read accross (RA)	%	8. IUCLID flags to omit the study (FO)	%	9. Weight of Evidence (WE)	%	10. QSAR Studies (QS)	%	11. Miscellaneous Studies (MS)	%
2 756	21.4	1 184	9.2	1 116	8.7	11	0.1	479	3.7
342	20.7	178	10.8	113	6.9	3	0.2	25	1.5
51	12.9	80	20.2	20	5.1	0	0.0	91	23.0
3 323	20.8	1 276	8	1 531	9.6	10	0.1	492	3.1
3 009	27.7	1 762	16.2	1 265	11.7	50	0.5	143	1.3
160	18	162	18.2	97	10.9	0	0.0	25	2.8
567	-0.6	92	-1.2	415	0.9	-1	0.0	13	-0.6
2 667	7	1 584	5.4	1 152	4.8	47	0.3	118	-0.2
109	5.1	82	-2	77	5.8	0	0.0	-66	-20.2
39	11.9	2	0.6	35	10.6	0	0.0	1	0.3
2	8.3	0	0.0	2	8.3	0	0.0	0	0.0
0	0	0	0.0	0	0.0	0	0.0	0	0.0
56	12.6	2	0.5	45	10.2	0	0.0	1	0.2
118	17.3	3	0.4	82	12.0	0	0.0	2	0.3
1	2.9	0	0.0	5	14.3	0	0.0	0	0.0
17	0.8	0	0.2	10	-0.5	0	0.0	0	-0.1
116	8.9	3	0.4	80	3.7	0	0.0	2	0.3
1	2.9	0	0.0	5	14.3	0	0.0	0	0.0
1 113	21.3	216	4.1	402	7.7	5	0.1	137	2.6
131	21.8	28	4.7	31	5.2	1	0.2	7	1.2
23	14.6	14	8.9	7	4.5	0	0.0	41	26.1
1 376	20.6	205	3.1	480	7.2	6	0.1	127	1.9
1 163	27.7	302	7.2	630	15.0	47	1.1	21	0.5
65	18.7	21	6.1	42	12.1	0	0.0	11	3.2
263	-0.7	-11	-1.1	78	-0.5	1	0.0	-10	-0.7
1 032	5.9	274	2.5	599	9.8	46	1.0	14	-0.7
42	4.1	7	-2.9	35	7.6	0	0.0	-30	-22.9
12	7	1	0.6	5	2.9	0	0.0	5	2.9
6	22.2	0	0.0	2	7.4	0	0.0	0	0.0
0	0	0	0.0	0	0.0	0	0.0	0	0.0
26	10.4	2	0.8	19	7.6	0	0.0	8	3.2
93	18.6	7	1.4	51	10.2	1	0.2	0	0.0
7	25	0	0.0	0	0.0	0	0.0	0	0.0
14	3.4	1	0.2	14	4.7	0	0.0	3	0.3
87	-3.6	7	1.4	49	2.8	1	0.2	0	0.0
7	25	0	0.0	0	0.0	0	0.0	0	0.0

1. Test type		2. Phase	3. Tonnage band	4. Total ESR	5. Experimental studis (ES)	%	6. Testing proposals (TP)	%
3.2 Eye irritation (*in vivo*)	2011	Phase-in	>1 000	4 221	2 714	64.3	0	0.0
		Phase-in	100 to 1 000	524	343	65.5	0	0.0
		Non-Phase-In	>1 00	140	63	45	0	0.0
	2014	Phase-in	>1 000	5 254	3 479	66.2	0	0.0
		Phase-in	100 to 1 000	3 691	1 861	50.4	0	0.0
		Non-Phase-In	>1 00	299	181	60.5	0	0.0
	Difference	Phase-in	>1 000	1 033	765	1.9	0	0.0
		Phase-in	100 to 1 000	3 167	1 518	-15.0	0	0.0
		Non-Phase-In	>1 00	159	118	15.5	0	0.0
4.1 Skin sensitisation (*in vitro*)	2011	Phase-in	>1 000	21	10	47.6	0	0.0
		Phase-in	100 to 1 000	4	4	100	0	0.0
		Non-Phase-In	>100	3	0	0.0	0	0.0
	2014	Phase-in	>1 000	14	10	71.4	0	0.0
		Phase-in	100 to 1 000	58	15	25.9	0	0.0
		Non-Phase-In	>100	3	1	33.3	0	0.0
	Difference	Phase-in	>1 000	-7	0	23.8	0	0.0
		Phase-in	100 to 1 000	54	11	-74.1	0	0.0
		Non-Phase-In	>100	0	1	33.3	0	0.0
4.2 Skin sensitisation (*in vivo*)	2011	Phase-in	>1 000	3 754	2 080	55.4	0	0.0
		Phase-in	100 to 1 000	488	283	58.0	0	0.0
		Non-Phase-In	>100	176	73	41.5	0	0.0
	2014	Phase-in	>1 000	4 657	2 566	55.1	0	0.0
		Phase-in	100 to 1 000	3 565	1 525	42.8	0	0.0
		Non-Phase-In	>100	299	198	66.2	0	0.0
	Difference	Phase-in	>1 000	903	486	-0.3	0	0.0
		Phase-in	100 to 1 000	3 077	1 242	-15.2	0	0.0
		Non-Phase-In	>100	123	125	24.7	0	0.0

	%	8. IUCLID flags to omit the study (FO)	%	9. Weight of Evidence (WE)	%	10. (Q)SAR studies (QS)	%	11. Miscellaneous studies (MS)	%
884	20.9	219	5.2	279	6.6	0	0.0	125	3
102	19.5	53	10.1	19	3.6	0	0.0	7	1.3
16	11.4	15	10.7	7	5	0	0.0	39	27.9
1 116	21.2	232	4.4	315	6	4	0.1	108	2.1
982	26.6	304	8.2	489	13.2	33	0.9	22	0.6
65	21.7	22	7.4	23	7.7	0	0.0	8	2.7
232	0.3	13	-0.8	36	-0.6	4	0.1	-17	-0.9
880	7.1	251	-1.9	470	9.6	33	0.9	15	-0.7
49	10.3	7	-3.4	16	2.7	0	0.0	-31	-25.2
6	28.6	0	0.0	5	23.8	0	0.0	0	0.0
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0	0.0	0	0.0	2	66.7	0	0.0	1	33.3
0	0.0	0	0.0	4	28.6	0	0.0	0	0.0
3	5.2	0	0.0	40	69.0	0	0.0	0	0.0
0	0.0	0	0.0	2	66.7	0	0.0	0	0.0
-6	-28.6	0	0.0	-1	4.8	0	0.0	0	0.0
3	5.2	0	0.0	40	69.0	0	0.0	0	0.0
0	0.0	0	0.0	0	0.0	0	0.0	-1	-33.3
782	20.8	264	7.0	513	13.7	18	0.5	97	2.6
119	24.4	0	0.0	72	14.8	3	0.6	11	2.3
27	15.3	35	19.9	4	2.3	0	0.0	37	21.0
1 059	22.7	275	5.9	621	13.3	29	0.6	107	2.3
1 029	28.9	254	7.1	678	19.0	52	1.5	27	0.8
50	16.7	15	5.0	29	9.7	2	0.7	5	1.7
277	1.9	11	-1.1	108	-0.3	11	0.1	10	-0.3
910	4.5	254	7.1	606	4.3	49	0.8	16	-1.5
23	1.4	-20	-14.9	25	7.4	2	0.7	-32	-19.4

Table 4: Human Health Endpoint Study Records: Dossiers subject to analysis in 2011 and 2014 (cut off date 1 October 2013)

1. Test type		2. Phase	3. Tonnage band	4. Total ESR	5. Experimental studis (ES)	%	6. Testing proposals (TP)	%
5.1 Genetic toxicity (*in vitro*)	2011	Phase-in	>1 000	10 322	5 908	57.2	0	0.0
		Phase-in	100 to 1 000	1 745	1 128	64.6	0	0.0
		Non-Phase-In	>1 000	351	180	51.3	0	0.0
	2014	Phase-in	>1 000	12 808	7 349	57.4	0	0.0
		Phase-in	100 to 1 000	10 083	4 267	42.3	0	0.0
		Non-Phase-In	>1 000	750	492	65.6	0	0.0
	Difference	Phase-in	>1 000	2 486	1 441	0.1	0	0.0
		Phase-in	100 to 1 000	8 338	3 139	-22.3	0	0.0
		Non-Phase-In	>1 000	399	312	14.3	0	0.0
5.2 Genetic toxicity (*in vivo*)	2011	Phase-in	>1 000	3 532	1 852	52.4	18	0.5
		Phase-in	100 to 1 000	596	366	61.4	2	0.3
		Non-Phase-In	>100	94	47	50.0	0	0.0
	2014	Phase-in	>1 000	4 281	2 222	51.9	15	0.4
		Phase-in	100 to 1 000	2 254	837	37.1	39	1.7
		Non-Phase-In	>100	180	103	57.2	4	2.2
	Difference	Phase-in	>1 000	749	370	-0.5	-3	-0.2
		Phase-in	100 to 1 000	1 658	471	-24.3	37	1.4
		Non-Phase-In	>100	86	56	7.2	4	2.2
6.0 Toxicity to reproduction	2011	Phase-in	>1 000	3 535	1 121	31.7	150	4.2
		Phase-in	100 to 1 000	487	146	30.0	9	1.8
		Non-Phase-In	>100	156	41	26.3	7	4.5
	2014	Phase-in	>1 000	4 508	1 309	29.0	159	3.5
		Phase-in	100 to 1 000	3 868	768	19.9	62	1.6
		Non-Phase-In	>100	327	87	26.6	9	2.8
	Difference	Phase-in	>1 000	973	188	-2.7	9	-0.7
		Phase-in	100 to 1 000	3 381	622	-10.1	53	-0.2
		Non-Phase-In	>100	171	46	0.3	2	-1.7
7.0 Developmental toxicity	2011	Phase-in	>1 000	4 217	1 783	42.3	151	3.6
		Phase-in	100 to 1 000	589	260	44.1	34	5.8
		Non-Phase-In	>100	121	36	29.8	13	10.7
	2014	Phase-in	>1 000	5 149	2 047	39.8	166	3.2
		Phase-in	100 to 1 000	4 216	858	20.4	293	6.9
		Non-Phase-In	>100	300	98	32.7	21	7.0
	Difference	Phase-in	>1 000	932	264	-2.5	15	-0.4
		Phase-in	100 to 1 000	3 627	598	-23.8	259	1.2
		Non-Phase-In	>100	179	62	2.9	8	-3.7



	%	8. IUCLID flags to omit the study (FO)	%	9. Weight of Evidence (WE)	%	10. (Q)SAR studies (QS)	%	11. Miscellaneous studies (MS)	%
2 272	22.0	394	3.8	1 245	12.1	5	0.0	498	4.8
308	17.7	53	3.0	206	11.8	0	0.0	50	2.9
36	10.3	32	9.1	10	2.8	1	0.3	92	26.2
2 910	22.7	374	2.9	1 604	12.5	6	0.0	565	4.4
3 073	30.5	371	3.7	2 210	21.9	92	0.9	70	0.7
170	22.7	38	5.1	40	5.3	1	0.1	9	1.2
638	0.7	-20	-0.9	359	0.5	1	0.0	67	-0.4
2 765	12.8	318	0.6	2 004	10.1	92	0.9	20	-2.2
134	12.4	6	-4.1	30	2.5	0	-0.2	-83	-25.0
875	24.8	221	6.3	389	11.0	0	0.0	177	5.0
128	21.5	26	4.4	60	10.1	0	0.0	14	2.3
5	5.3	7	7.4	1	1.1	0	0.0	34	36.2
1 076	25.1	214	5.0	490	11.4	1	0.0	263	6.1
693	30.7	128	5.7	506	22.4	25	1.1	26	1.2
42	23.3	18	10.0	10	5.6	0	0.0	3	1.7
201	0.4	-7	-1.3	101	0.4	1	0.0	86	1.1
565	9.3	102	1.3	446	12.4	25	1.1	12	-1.2
37	18.0	11	2.6	9	4.5	0	0.0	-31	-34.5
840	23.8	904	25.6	428	12.1	4	0.1	88	2.5
118	24.2	138	28.3	47	9.7	0	0.0	29	6.0
11	7.1	64	41.0	6	3.8	0	0.0	27	17.3
1 258	27.9	1 052	23.3	611	13.6	4	0.1	115	2.6
1 005	26.0	1 365	35.3	605	15.6	40	1.0	23	0.6
58	17.7	129	39.4	40	12.2	0	0.0	4	1.2
418	4.1	148	-2.2	183	1.4	0	0.0	27	0.1
887	1.8	1 227	7.0	558	6.0	40	1.0	-6	-5.4
47	10.7	65	-1.6	34	8.4	0	0.0	-23	-16.1
1 254	29.7	460	10.9	451	10.7	7	0.2	111	2.6
174	29.5	71	12.1	32	5.4	2	0.3	16	2.7
12	9.9	40	33.1	4	3.3	0	0.0	16	13.2
1 619	31.4	551	10.7	610	11.8	7	0.1	149	2.9
1 582	37.5	668	15.8	745	17.7	40	0.9	30	0.7
73	24.3	74	24.7	32	10.7	0	0.0	2	0.7
365	1.7	91	-0.2	159	1.2	0	0.0	38	0.3
1 408	8.0	597	3.8	713	12.2	38	0.6	14	-2.0
61	14.4	34	-8.4	28	7.4	0	0.0	-14	-12.6

1. Test type		2. Phase	3. Tonnage band	4. Total ESR	5. Experimental studis (ES)	%	6. Testing proposals (TP)	%
7.7 Carcinogenicity	2011	Phase-in	>1 000	3 559	1 377	38.7	2	0.1
		Phase-in	100 to 1 000	451	254	56.3	1	0.2
		Non-Phase-In	>1 000	29	4	13.8	0	0.0
	2014	Phase-in	>1 000	4 088	1 566	38.3	2	0.0
		Phase-in	100 to 1 000	1 299	427	32.9	0	0.0
		Non-Phase-In	>1 000	94	11	11.7	0	0.0
	Difference	Phase-in	>1 000	529	189	-0.4	0	0.0
		Phase-in	100 to 1 000	848	173	-23.4	-1	-0.2
		Non-Phase-In	>1 000	65	7	-2.1	0	0.0
9.0 Repeated dose toxicity (all routes)	2011	Phase-in	>1 000	10 790	4 546	42.1	104	1.0
		Phase-in	100 to 1 000	1 333	538	40.4	32	2.4
		Non-Phase-In	>100	359	105	29.2	8	2.2
	2014	Phase-in	>1 000	13 038	5 503	42.2	138	1.1
		Phase-in	100 to 1 000	9 786	2 411	24.6	227	2.3
		Non-Phase-In	>100	870	296	34.0	12	1.4
	Difference	Phase-in	>1 000	2 248	957	0.1	34	0.1
		Phase-in	100 to 1 000	8 453	1 873	-15.7	195	-0.1
		Non-Phase-In	>100	511	191	4.8	4	-0.8

	%	8. IUCLID flags to omit the study (FO)	%	9. Weight of Evidence (WE)	%	10. (Q)SAR studies (QS)	%	11. Miscellaneous studies (MS)	%
992	27.9	530	14.9	434	12.2	7	0.2	217	6.1
100	22.2	59	13.1	26	5.8	0	0.0	11	2.4
7	24.1	14	48.3	0	0.0	0	0.0	4	13.8
1 118	27.3	622	15.2	502	12.3	7	0.2	271	6.6
438	33.7	184	14.2	211	16.2	8	0.6	31	2.4
29	30.9	38	40.4	15	16.0	0	0.0	1	1.1
126	-0.5	92	0.3	68	0.1	0	0.0	54	0.5
338	11.5	125	1.1	185	10.5	8	0.6	20	-0.1
22	6.7	24	-7.9	15	16.0	0	0.0	-3	-12.7
3 032	28.1	2 033	18.8	709	6.6	9	0.1	357	3.3
355	26.6	262	19.7	101	7.6	0	0.0	45	3.4
30	8.4	162	45.1	3	0.8	0	0.0	51	14.2
3 510	26.9	2 453	18.8	1 051	8.1	11	0.1	372	2.9
3 220	32.9	2 435	24.9	1 372	14.0	36	0.4	85	0.9
165	19.0	321	36.9	68	7.8	1	0.1	7	0.8
478	-1.2	420	0.0	342	1.5	2	0.0	15	-0.5
2 865	6.3	2 173	5.2	1 271	6.4	36	0.4	40	-2.5
135	10.6	159	-8.2	65	7.0	1	0.1	-44	-13.4

Tables 3 and 4 summarise the information provided for the toxicological REACH information requirements normally to be satisfied with studies using vertebrate animals. By comparing the situation at the time of the last report (2011), certain trends could be observed.

For the **acute toxicity** endpoint, relatively less frequent information from experimental studies is provided for phase-in substances (-17%) while the use of adaptations (read-across, weight of evidence, omission of experimental data) has increased accordingly (+17%). For non phase-in substances the frequency for recording experimental studies increased (+11%) as well as the number of adaptations (+11%). The amount of information from unidentified sources decreased accordingly (-20%). See Appendix 1 for a more detailed analysis.

The information requirement for *in vivo* **skin irritation** was also addressed relatively more frequently using adaptations. Table 3 discriminates *in vivo* and *in vitro* experimental studies. The total number of ESRs submitted for *in vitro* skin irritation has substantially increased (273 ESRs in the last report and 846 ESRs in the current data pool). The increased use of *in vitro* methods for this endpoint is not visible in the relative percentages calculated due to the reasons that, for example, in the previous report only 218 phase-in substances at 100 to 1 000 tonnes per year were analysed and the number of the phase-in dossiers analysed in this report has substantially increased (1 870). The relative comparison of these two entries reveals that in non-phase-in dossiers, *in vivo* studies have been recorded more frequently than previously (+14%) in contrast to *in vitro* studies (-17%). For phase-in substances the picture changes with a reduction in the relative number of experimental study records (-13% and -16%) while adaptations (read-across, weight of evidence, omission of experimental data) are used more frequently (+13% and +16%). More detailed analysis of the data and dossiers regarding how the registrants used various approaches to cover information requirements for this endpoint is provided in Appendix 2.

Increased use of *in vitro* tests for the information requirement of *in vivo* eye irritation can be explained by the availability of new OECD Test Guidelines, as described in Appendix 13. The total number of ESRs submitted for *in vitro* **eye irritation** has substantially increased (169 ESRs in the last report and 564 ESRs in the current data pool). The increased use of *in vitro* methods for this endpoint is not visible in the relative percentages calculated due to the reasons that, for example, in the previous report only 218 phase-in substances at 100 to 1 000 tonnes per year were analysed and the number of those phase-in dossier analysed in this report has substantially increased (1 870). The relative comparison of non phase-in registrations shows a similar picture as for skin irritation (here 25% *in vitro*, +16% *in vivo*). Noticeable relative increases on the use of read-across and of weight of evidence approaches for this endpoint are present in Table 3. As for the skin irritation, more detailed analysis of the data and dossiers regarding how the registrants used various approaches to cover information requirements for eye irritation is provided in Appendix 3.

Two mostly noticeable differences in registrants' behaviour regarding **skin sensitisation *in vitro*** for Annex IX dossiers are 1) increased total number of ESRs (58 submitted by 1 October 2013, four in 2011, respectively) and a relative growth of the use of weight of evidence (40 ESR in the current data pool, or 69% more than in 2011). More experimental studies have been used in the current data pool to cover this endpoint for the substances of the highest tonnage band (71.4% of ESRs in the current data pool, while only 47.6% of ESRs in previously analysed dossiers). When fulfilling standard information requirements for **skin sensitisation *in vivo***, the relative percentage use of experimental studies dropped in Annex IX dossiers (58.0% of ESRs in 2011 and 42.8% in the current data pool, respectively) but grew for the non-phase-in substances (almost +25% of the relative difference). Due to more in-depth analysis of the use of alternatives also being conducted for this endpoint, detailed data are provided in Appendix 4.

Regarding **genetic toxicity *in vitro*** and ***in vivo***, both in 2011 and in the current data pool registrants seemed to use similar approaches for covering information requirements by experimental studies or using alternative methods. Despite a remarkable increase in the total number of ESRs submitted by 1 October 2013, the use of experimental data dropped for both Annex IX and Annex X dossiers (see rows 5.1 and 5.2 of Table 4), while a relative increase in the

use of a read-across approach (up to almost +13% for Annex IX dossiers on phase-in substances) and less expressed – weight of evidence (+10% relative increase for the same tonnage band) was identified for **genetic toxicity *in vitro***. Similar trends could be observed for vertebrate testing, where a relative frequence of the use of read-across was 18% higher for non-phase-in substances from the current data pool. For a more detailed analysis, consult Appendix 6.

After acute toxicity, the **repeated dose toxicity** endpoint was found to be the second most ESR-rich endpoint in the current data pool (total numbers provided in row 9 of Table 4). The results demonstrate that this is also the second endpoint regarding the number of submitted testing proposals (227 new studies proposed for the Annex IX dossiers submitted by 1 October 2013). A relative decrease of the use of experimental data was also observed for this tonnage band (-15.7% when compared with the data of 2011). As for all other higher tier endpoints, the registrants seemed more often to apply read-across and weight of evidence approaches, while for non-phase-in substances a relatively smaller percentage of ESRs contained a proposal to omit the study. In addition, for these substances, both absolute and relative decrease of use of other (miscellaneous data) was noted. See Appendix 5 for a more detailed analysis.

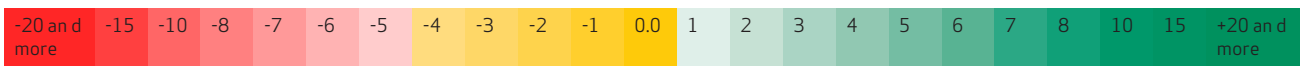
When analysing the ESR content in dossiers submitted for phase-in substances manufactured between 100 and 1 000 tonnes per year in the current data pool, it appears that the registrants tended to decrease the use of experimental tests for **toxicity to reproduction** (-10% relative decrease). Regarding the dossiers falling under the highest tonnage band, there were no significant differences but an increased absolute number of ESRs was observed. In contrast, when analysing dossiers of non-phase-in substances, clear trends of an increased use of read-across (+10.7% relative increase) and of weight of evidence approaches (+8.4% relative increase) were noted.

Findings with regards to the **pre-natal developmental toxicity** endpoint shared a similar tendency: even though the total amount of entries has grown substantialy, relatively, the registrants covered much fewer ESRs by studies conducted on vertebrate animals (-23% relative percentage decrease noted for Annex IX dossiers). This might partially be explained by the fact that this endpoint was among those for which the registrants submitted testing proposals by the 31 May 2013 registration deadline (293 ESRs found). A relative increase of use of the most common alternative approaches, such as read-across and weight of evidence, was also noted for both phase-in substances registered under Annex IX and for non-phase-in substances. See Appendix 7 for a more detailed analysis.

Relatively similar trends of the distribution of means to cover endpoints were also noted for **carcinogenicity**. This endpoint, however, is not a standard information requirement under REACH; the relative use of experimental data dropped in Annex IX dossiers (-23.4%) almost as much as for pre-natal developmental toxicity, while more endpoints were covered by read-across and weight of evidence for both phase-in substances registered under Annex IX and for non-phase-in substances. See Appendix 8 for a more detailed analysis.

Table 5: Environment Endpoint Study Records: Dossiers subject to analysis in 2011 and 2014 (cut off date 1 October 2013)

1. Test type	2. Phase	3. Tonnage band	4. Total ESR	5. Experimental Studies (ES)	%	6. Testing Proposals (TP)	%
2010 Bioaccumulation (fish)	Phase-in	>1 000	798	336	42.1	12	1.5
	Phase-in	100 to 1 000	278	59	21.2	5	1.8
	Non-Phase-In	>100	20	14	70.0	0	0.0
2014 Bioaccumulation (fish)	Phase-in	>1 000	1 854	408	22.0	6	0.3
	Phase-in	100 to 1 000	1 741	226	13.0	9	0.5
	Non-Phase-In	>100	124	45	36.3	0	0.0
Difference	Phase-in	>1 000	1 056	72	-20.1	-6	-1.2
	Phase-in	100 to 1 000	1 463	167	-8.2	4	-1.3
	Non-Phase-In	>100	104	31	-33.7	0	0.0
2010 Short-term toxicity to fish	Phase-in	>1 000	6 942	3 653	52.6	0	0.0
	Phase-in	100 to 1 000	1 405	684	48.7	0	0.0
	Non-Phase-In	>100	143	76	53.1	0	0.0
2014 Short-term toxicity to fish	Phase-in	>1 000	8 917	4 552	51.0	0	0.0
	Phase-in	100 to 1 000	6 104	2 368	38.8	0	0.0
	Non-Phase-In	>100	362	213	58.8	0	0.0
Difference	Phase-in	>1 000	1 975	899	-1.6	0	0.0
	Phase-in	100 to 1 000	4 699	1 684	-9.9	0	0.0
	Non-Phase-In	>100	219	137	5.7	0	0.0
2010 Long-term toxicity to fish	Phase-in	>1 000	3 281	899	27.4	27	0.8
	Phase-in	100 to 1 000	812	288	35.5	10	1.2
	Non-Phase-In	>1 000	101	14	13.9	0	0.0
2014 Long-term toxicity to fish	Phase-in	>1 000	4 041	1 200	29.7	19	0.5
	Phase-in	100 to 1 000	3 563	420	11.8	25	0.7
	Non-Phase-In	>1 000	225	32	14.2	1	0.4
Difference	Phase-in	>1 000	760	301	2.3	-8	-0.3
	Phase-in	100 to 1 000	2 751	132	-23.7	15	-0.5
	Non-Phase-In	>1 000	124	18	0.3	1	0.4
2010 Long-term toxicity to birds	Phase-in	>1 000	2 007	216	10.8	4	0.2
	Phase-in	100 to 1 000	350	57	16.3	0	0.0
	Non-Phase-In	>100	36	0	0.0	0	0.0
2014 Long-term toxicity to birds	Phase-in	>1 000	2 435	285	11.7	1	0.0
	Phase-in	100 to 1 000	975	123	12.6	0	0.0
	Non-Phase-In	>100	104	13	12.5	0	0.0
Difference	Phase-in	>1 000	428	69	0.9	-3	-0.2
	Phase-in	100 to 1 000	625	66	-3.7	0	0.0
	Non-Phase-In	>100	68	13	12.5	0	0.0



7. Read-across (RA)	%	8. IUCLID flags to omit the study (FO)	%	9. Weight of Evidence (WE)	%	10. QSAR Studies (QS)	%	11. Miscellaneous Studies (MS)	%
197	24.7	0	0.0	204	25.6	25	3.1	24	3.0
103	37.1	0	0.0	107	38.5	0	0.0	3	1.1
3	15.0	0	0.0	1	5.0	0	0.0	2	10.0
247	13.3	0	0.0	418	22.5	723	39.0	52	2.8
298	17.1	0	0.0	992	57.0	193	11.1	23	1.3
13	10.5	0	0.0	27	21.8	39	31.5	0	0.0
50	-11.4	0	0.0	214	-3.0	698	35.9	28	-0.2
195	-19.9	0	0.0	885	18.5	193	11.1	20	0.2
10	-4.5	0	0.0	26	16.8	39	31.5	-2	-10.0
1 400	20.2	124	1.8	983	14.2	147	2.1	635	9.1
384	27.3	12	0.9	227	16.2	18	1.3	80	5.7
12	8.4	6	4.2	6	4.2	3	2.1	40	28.0
1 692	19.0	103	1.2	1 335	15.0	330	3.7	905	10.1
2 154	35.3	131	2.1	1 094	17.9	120	2.0	237	3.9
82	22.7	7	1.9	38	10.5	12	3.3	10	2.8
292	-1.2	-21	-0.6	352	0.8	183	1.6	270	1.0
1 770	8.0	119	1.3	867	1.8	102	0.7	157	-1.8
70	14.3	1	-2.3	32	6.3	9	1.2	-30	-25.2
697	21.2	1 113	33.9	296	9.0	141	4.3	108	3.3
282	34.7	139	17.1	67	8.3	10	1.2	16	2.0
3	3.0	66	65.3	6	5.9	2	2.0	10	9.9
860	21.3	1 206	29.8	480	11.9	171	4.2	105	2.6
1 025	28.8	1 515	42.5	462	13.0	58	1.6	58	1.6
20	8.9	142	63.1	24	10.7	6	2.7	0	0.0
163	0.0	93	-4.1	184	2.9	30	-0.1	-3	-0.7
743	-6.0	1 376	25.4	395	4.7	48	0.4	42	-0.3
17	5.9	76	-2.2	18	4.7	4	0.7	-10	-9.9
128	6.4	1 460	72.7	198	9.9	1	0.0	0	0.0
36	10.3	145	41.4	101	28.9	0	0.0	11	3.1
0	0.0	33	91.7	1	2.8	0	0.0	2	5.6
159	6.5	1 637	67.2	309	12.7	1	0.0	43	1.8
198	20.3	504	51.7	132	13.5	1	0.1	17	1.7
1	1.0	81	77.9	9	8.7	0	0.0	0	0.0
31	0.2	177	-5.5	111	2.8	0	0.0	43	1.8
162	10.0	359	10.3	31	-15.3	1	0.1	6	-1.4
1	1.0	48	-13.8	8	5.9	0	0.0	-2	-5.6

As seen from Table 5, a total of 3 719 ESRs reporting vertebrate species have been identified for the **bioaccumulation** endpoint, against 1 096 of the previous registration deadline. For substances between 100 and 1 000 tonnes per year, the main approach used was weight of evidence (57.0% on a total pool of 1 741 ESRs), followed by read-across (17.1%). For the former, this represents a relative increase of 18.5%; for the latter, a decrease of 19.9% compared to 2011. In the highest tonnage band, i.e. at or above 1 000 tonnes per year, (Q)SARs represented the preferred option with a total of 723 ESRs out of a total pool of 1 854, i.e. 39% of the hits. Weight of evidence is the second most used approach (22.5%) also at this tonnage band, closely followed by experimental data entries (22.0% of the ESRs, representing a relative reduction of 20.1% compared to 2011). The value for the (Q)SARs represents a 35.9% relative increase compared to the previous registration deadline. However, a detailed analysis has revealed that this number is strongly influenced by a group of UVCB substances contained in the database. See Appendix 9 for a more detailed analysis.

Under the aquatic toxicity endpoints, **short-term toxicity to fish** counted for a total of 15 383 ESRs. The core of the entries was distributed between experimental data entries and read-across for all the three types of registrations, i.e. phase-in substances between 100 and 1 000 tonnes per year, those at or above 1 000 tonnes per year and non-phase-in substances. Respectively, the experimental studies resulted in 2 368 ESRs (38.8% of the total for this tonnage band), 4 552 ESRs (51.0%) and 213 ESRs (58.8%); whereas the read-across approach resulted in 2 154 ESRs (35.3%), 1 692 ESRs (19.0%) and 82 ESRs (22.7%).

The 'sister' endpoint to the above in the aquatic toxicity compartment is **long-term toxicity to fish**. The total number of ESRs has increased from 4 194 in 2011 to 7 829 submitted by 1 October 2013. The distribution among the various approaches chosen is comparable between the two registration deadlines, with differences of a maximum of 10% except for the use of some approaches, i.e. use experimental studies and proposals to omit the study for phase-in substances between 100 and 1 000 tonnes per year. In fact, the 420 entries for experimental studies on a total of 3 563 represent a 23.7% drop when compared to the respective figure for 2011.

Similarly, the registrants proposal to omit the study resulted in 1 515 entries compared to the 139 of 2011, i.e. a relative increase of 25.4%. See Appendix 10 for a more detailed analysis.

Last, but not least, ECHA has analysed the results regarding **long-term or reproductive toxicity to birds**. This endpoint is not a standard information requirement under REACH. As it is transparent from Figure 11.1 in Appendix 11, the figures relative to the differences in percentage of entries between the two registration deadlines indicate both plus and minus signs, thus showing no significant trend. Nevertheless, on a total of 2 393 ESRs in 2011 and 3 478 submitted by 1 October 2013, it can be noticed that the highest proportion of hits in all three types of registrations has been found under proposals to omit the study (1 637 for Annex X substances, 504 for Annex IX and 81 for non-phase-in). See Appendix 11 for a more detailed analysis.

3.3 SUBSTANCE APPROACH

As with the previous report, it is of further interest to analyse at substance level, how the registrants used alternative approaches. Such analysis gives the relative proportions of the main options used by registrants to fill the information requirements for each endpoint per registered substance. In this case, the data pool represents 3 662 substances (both phase-in and non-phase-in) manufactured or imported at or above 100 – 1 000 and at or above 1 000 tonnes per year.

In 2011, for this analysis, ECHA generally categorised the approaches used by the registrants as testing proposals, experimental studies and alternative methods only. In 2014, to provide a more detailed overview, the options used were further split as presented for the ESR approach. The other alternatives to animal testing referred to either weight of evidence (WE), (Q)SARs (QS), read-across (RA) or other approaches such as proposals to omit the study (FO) or no data.

Consistent with the analysis in the first report, ECHA assumed:

- If there was a testing proposal included, this was taken as evidence that the endpoint was supposed to be filled by future testing;

- If there was at least one weight of evidence ESR included, this was taken as evidence that the endpoint was supposed to be filled by a weight of evidence approach;
- If there was one ESR entry referring to an experimental study, this was taken as evidence that the endpoint on the substance level was filled with experimental data (excluding a weight of evidence approach also using experimental data: if an experimental study was found in parallel with a weight of evidence approach, it was considered and reported only as a weight of evidence approach); and
- If there was no ESR entry referring to an experimental study but listing either a possibility to omit the information or to fill the information requirements using alternative approaches, it was counted as evidence that the endpoint on the substance level was filled with an alternative method. Alternative methods used by the registrants were identified in the following order: (Q)SARs, read-across, proposals to omit the study.

Each of these options has only been counted once for each endpoint at substance level. Therefore, this way of analysing the data does not provide a frequency distribution on how many experimental or alternative studies have been entered for each endpoint at substance level.

For some endpoints, the need to address an information requirement is dependent on the findings from other endpoints. In such cases, there is no obligation for the registrant to enter information into IUCLID dossiers. Such situations are characterised by "not reported" (NR).

Considering there were no major changes in the standard information requirements under the REACH Regulation, ECHA did not expect major changes in the registrants' behaviour except for certain endpoints, as outlined above under the ESR approach.

This analysis provides an overall relationship between experimental studies and alternative options for the REACH-relevant endpoints. The experimental studies have been counted for each substance without checking the study type or the quality of the information for the endpoints.

Therefore, it is important to note that an entry as experimental study under an endpoint does not mean that the information requirement has been filled according to the requirements in the REACH Annexes. The percentages shown in the bar chart represent an upper boundary for experimental data availability for the endpoints, excluding the cases in which experimental data were provided as a part of a weight of evidence approach.

When analysing the current data pool, experimental studies were available to cover **acute toxicity** for 70% of substances – experimental data richest endpoint. The second choice to fulfil information requirements was the use of a weight of evidence approach (18%), followed by read-across (10%). For **acute toxicity**, testing proposals are not used for this endpoint since it is an Annex VII and VIII standard information requirement. Studies were omitted for only 1% of the analysed substances.

Regarding **skin corrosion/irritation**, as presented in Figure 2, in 69% of the cases the endpoint was filled with experimental data, while in 15% of the cases registrants chose to apply read-across followed by a weight of evidence approach (11%). Similar approaches were taken by the registrants with regard to **eye irritation**: experimental studies were available for 67% of analysed substances, followed by use of a read-across approach (16%) and weight of evidence (11%).

The registrants submitted experimental studies to fulfil information requirements for **skin sensitisation** for 55% of the analysed substances. Read-across was the second most frequently chosen option (23%) while registrants proposed to use a weight of evidence approach for 12% of substances. In 9% of all cases, the studies were omitted.

The **genetic toxicity *in vitro*** endpoint was covered by experimental data in 63% of the cases while alternative options were used for the remaining cases (18% by weight of evidence and 16% using a read-across approach, respectively). In contrast to the *in vitro* studies, experimental data were only available to cover 43% of the cases of **genetic toxicity *in vivo***. This might be because *in vivo* tests may not need to be conducted for this endpoint, depending on the results of the *in vitro* studies. In 32% of the cases, the read-across option to fulfil

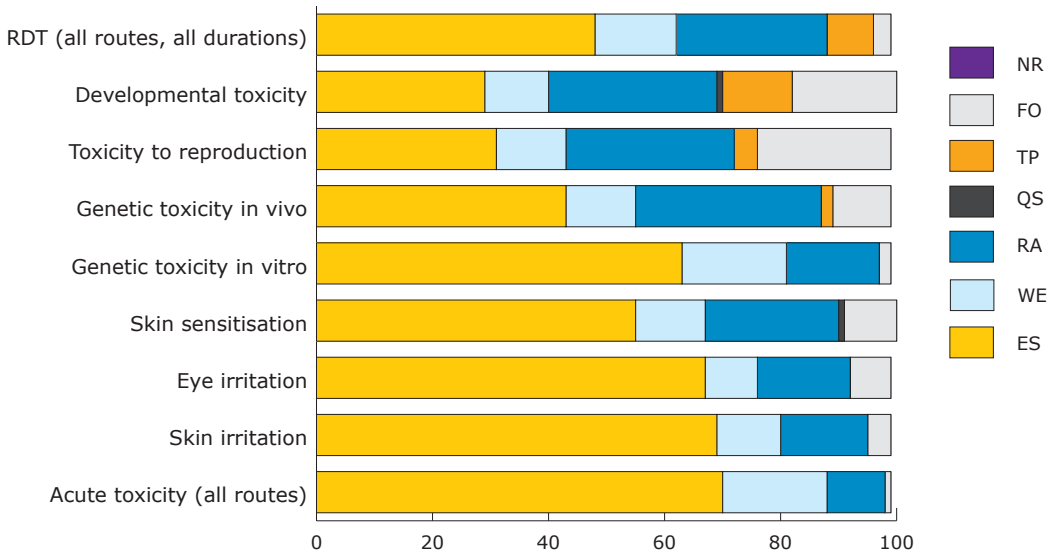


Figure 2. Relative proportions of the principal options to fulfil information requirements for human health endpoints for the substances (phase-in, at or above 100 tonnes per year and at or above 1 000 tonnes per year, 3 662 substances)

Legend
ES – Experimental studies
WE – Weight of evidence
RA – Read-across
QS – (Q)SAR
TP – Testing proposal
FO – Flags to omit study
NR – Not reported

standard information requirements was chosen, while a weight of evidence approach was proposed for 12% of the cases. The registrants submitted testing proposals to cover this endpoint for 2% of the analysed substances. For every 10th substance, registrants chose to omit the test.

The **repeated dose toxicity** endpoint (addressing all routes and all durations of studies) was covered by experimental studies for 48% of the analysed substances. In 8% of the cases, registrants submitted testing proposals for this endpoint and 26% of the entries chosen were covered by a read-across approach. Weight of evidence was used for 14% of the substances and 3% of the entries proposed to omit the study.

As already explained in the previous report, availability of experimental data for **toxicity to reproduction** and **pre-natal developmental toxicity** does not mean that the information requirements are filled according to the requirements in the REACH Annexes. As presented in Figure 2, almost 31% of the analysed substances at or above 100–1 000 tonnes per year and at or above 1 000 tonnes per year already had experimental data on toxicity to reproduction, while in 29% of the cases registrants used the read-across option to cover the endpoint, following by use of weight of evidence (12%).

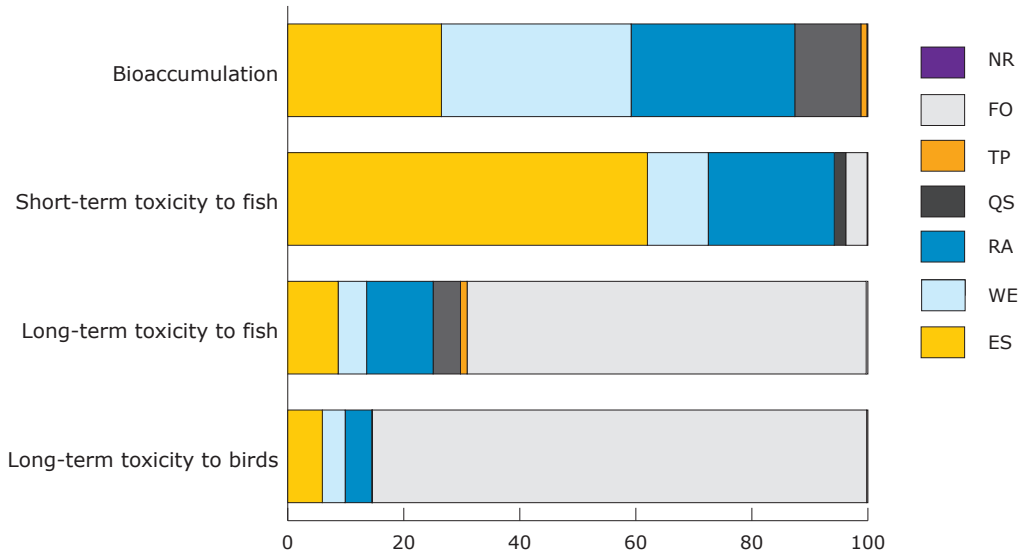


Figure 3. Relative proportions of the principal options to fulfil information requirements for environmental endpoints for the substances (phase-in, at or above 100 tonnes per year and at or above 1 000 tonnes per year, 3 662 substances)

Legend
ES – Experimental studies
WE – Weight of evidence
RA – Read-across
QS – (Q)SAR
TP – Testing proposal
FO – Flags to omit study
NR – Not reported

In contrast with the first report, testing proposals for toxicity to reproduction (usually, two-generation reproductive toxicity study) were submitted for only 4% of the analysed substances, while proposals to omit the study were chosen in 23% of the cases. This can be explained by different standard information requirements for substances manufactured or imported at or above 100 tonnes per year and for the ones produced at or above 1 000 tonnes per year. For the lower tonnage grade, two-generation reproductive toxicity study is only required if the sub-acute (28-day study) or sub-chronic (90-day study) indicate adverse effects on reproductive organs or tissues.

Experimental **pre-natal developmental toxicity** data were available for 29% of the substances, while in another 29% of the cases registrants used a read-across approach to make their dossiers complete. There were more tests proposed than for toxicity to reproduction (12% of cases). Similarly, registrants chose to omit the test(s) for 18% of the substances.

Experimental data on **bioaccumulation in fish,** as presented in Figure 3, were available for 26.5% of the analysed substances. For 1% of the substances, testing proposals were submitted. For more than two thirds of the substances, registrants used alternative options to cover this endpoint (almost equally spread between weight of evidence (32.7%)

and read-across (28.2%) and less using (Q)SARs (11.4%). The experimental data on invertebrates have been counted as alternative methods for the purpose of this report. No data were reported in 0.2% of cases.

With regard to **short-term toxicity to fish**, experimental studies were available for 62% of the cases and the study was omitted in 3.7% of the cases. Registrants used various alternative options to cover the remaining 34.2% of the entries, mostly by read-across (21.7%), weight of evidence (10.5%) and in 2% of cases by using (Q)SARs. In 0.1% of the cases, no data were reported.

For **long-term toxicity to fish**, registrants submitted experimental data for less than 8.7% of the covered substances and submitted the testing proposals for 1.1%. Alternative options were used for 21.1%, while the registrant omitted the study in 68.8% of cases. Read-across was used for this endpoint in 11.5% of cases. Weight of evidence was used in 5% of the cases and 4.7% by using (Q)SAR. In 0.3% of the cases, no data were reported.

For the endpoint **long-term toxicity to birds**, information might be required under Annex X. However, as outlined above, this is not a standard information requirement. Experimental data covered only 6% of the selected substances while in 85% of the cases, registrants submitted justifications to omit the study to cover this endpoint. The registrants also used the alternative methods to cover this endpoint (4% by weight of evidence, 4.6% by read-across and 0.09% by (Q)SARs. In 0.2% of the cases, no data were reported.

3.4 ANALYSIS OF ADAPTATIONS MADE ACCORDING TO REACH ANNEX XI

The structure of the information submitted by registrants in IUCLID is very complex, often having sophisticated relationships between different data entries in the registration dossier. Therefore, to present a comprehensive view of these data, more than one perspective of the data is needed. The purpose of this section is therefore to describe how the Agency has performed in-depth analysis of adaptations used by the registrants according to the general rules, laid down in Annex XI of the REACH Regulation.

3.4.1 Adaptations analysed

The analysis done as described in this part of the report focuses on the following types of adaptations: read-across, categories, (Q)SARs, and weight of evidence. *In vitro* methods are analysed separately because there are regulatory accepted *in vitro* alternatives already but only for a limited number of endpoints.

In this analysis, specific data mining techniques were used that are completely different from the analyses done and described in other chapters of this report. Therefore, the results of this chapter should not be compared with the results of any other chapter of this report. Equivalent analyses have not been performed for the first report published in 2011. Thus, a comparison of these results with any results from the previous report is not possible.

3.4.2 Methodology

ECHA applied a similar workflow as for the endpoint study record (ESR) analysis (see sections 3.1 and 3.2 of this report). More specifically, read-across was analysed in two different perspectives, which do not overlap. The first perspective takes into account the read-across cases submitted without using the IUCLID category template. The second perspective focuses on those read-across or grouping instances submitted using the IUCLID category template.

In addition to read-across and (Q)SARs, the Agency analysed the type of data that are included in the weight of evidence (WE) approaches. In the context of weight of evidence, several alternative methods might be used simultaneously. These were counted regardless of the context indicated by the purpose flag in the IUCLID dossier. For every endpoint, a separate analysis of the ESR distribution per purpose flag was performed. The detailed results from these analyses are presented in Appendix 12 of this report. In these tables, the purpose flag could be "key study", "supporting study", "weight of evidence", or "not assigned". Thus, the distribution of the ESR according to purpose flag per endpoint always totals 100%.

There could potentially be overlaps between alternatives for endpoints, where the adaptations from different approaches are combined by the registrants. For example, a read-across approach may have been supported by (Q)SAR prediction, or vice versa. The algorithm set for this report analyses the scenario separately, when the registrant uses the purpose flag "weight of evidence" in the IUCLID study result type field. The testing proposals are not analysed here as they are considered beyond the scope of this analysis. Consequently, this approach disregards any endpoint study record with "experimental study planned" as the IUCLID study result type. In the selection of ESRs for this part of the analysis, the disregarded studies (as flagged by the registrant as such by the purpose flag) were removed. Whenever a category template was used (either on an endpoint or at dossier level), the ESRs were also analysed separately.

The data pool for the first perspective was the same as for the ESR approach. ECHA analysed 3 813 lead and individual dossiers covering 3 662 substances at or above 100 tonnes per year (ref. Table 2 of Section 3.1 of this report). For the second perspective, only those dossiers, which were submitted in the IUCLID category template and therefore excluded from the main data pool, were analysed. This second pool consisted of 649 IUCLID category dossiers (533 lead and individual dossiers, 116 member dossiers) covering 523 substances at or above 100 tonnes per year, covering 121 different categories, as defined by the registrants.

Figure 4 below illustrates the algorithm used for data extraction.

3.4.3 Unique Experimental Study (UES) concept

More detailed analysis on read-across and categories required the possibility to remove the duplicate endpoint study records from the analysis and counting. This need has led to the development and implementation of the unique experimental study (UES) concept. The UES concept was introduced since the same ESRs might have been used in several category dossiers. Hence, by applying a unique identifier concept, it is possible to avoid double counting.

For all experimental and read-across studies, a content fingerprint has been created for each ESR by concatenating the content from selected fields. The fingerprints were created using information from the following sections of the ESRs:

- Administrative data;
- Data source;
- Materials and methods;
- Test animals/Test organism;
- Results and discussion;
- Applicant's summary and conclusions.

The UES can be identified by matching fingerprints from all the ESRs reported by registrants as an experimental study. The experimental studies are counted only once regardless of the number of ESRs where they were reported. Figure 5 schematically presents the general process of building the fingerprints for identifying UESs. Not all fields that participate in the fingerprint calculation could be shown on the plot.

To assess the potential of avoiding unnecessary testing by building categories (by using the IUCLID category template), ECHA introduced the ESR substitution ratio for category (CAT) dossiers:

$$\textit{ESR Substitution Ratio [CAT]} = \frac{\#ESR_{[Exp]} + \#ESR_{[RA^*]}}{\#UES_{[CAT]}}$$

Where:

$\#ESR_{[Exp]}$ - Number of all ESRs flagged by registrant as experimental result in the category (CAT) dossiers,

$\#ESR_{[RA^*]}$ - Number of ESRs flagged by registrant as read-across where the content fingerprint is identical to at least one experimental ESR in the CAT dossiers.

$\#UES_{[Exp+RA^*]}$ - Number of unique experimental studies identified for all experimental ESRs in CAT dossiers.

The UESs are able to identify unique studies regardless of how the registrants have reported their category in IUCLID. To allow comparison between category and non-category dossiers, the same fingerprint approach was also applied to non-

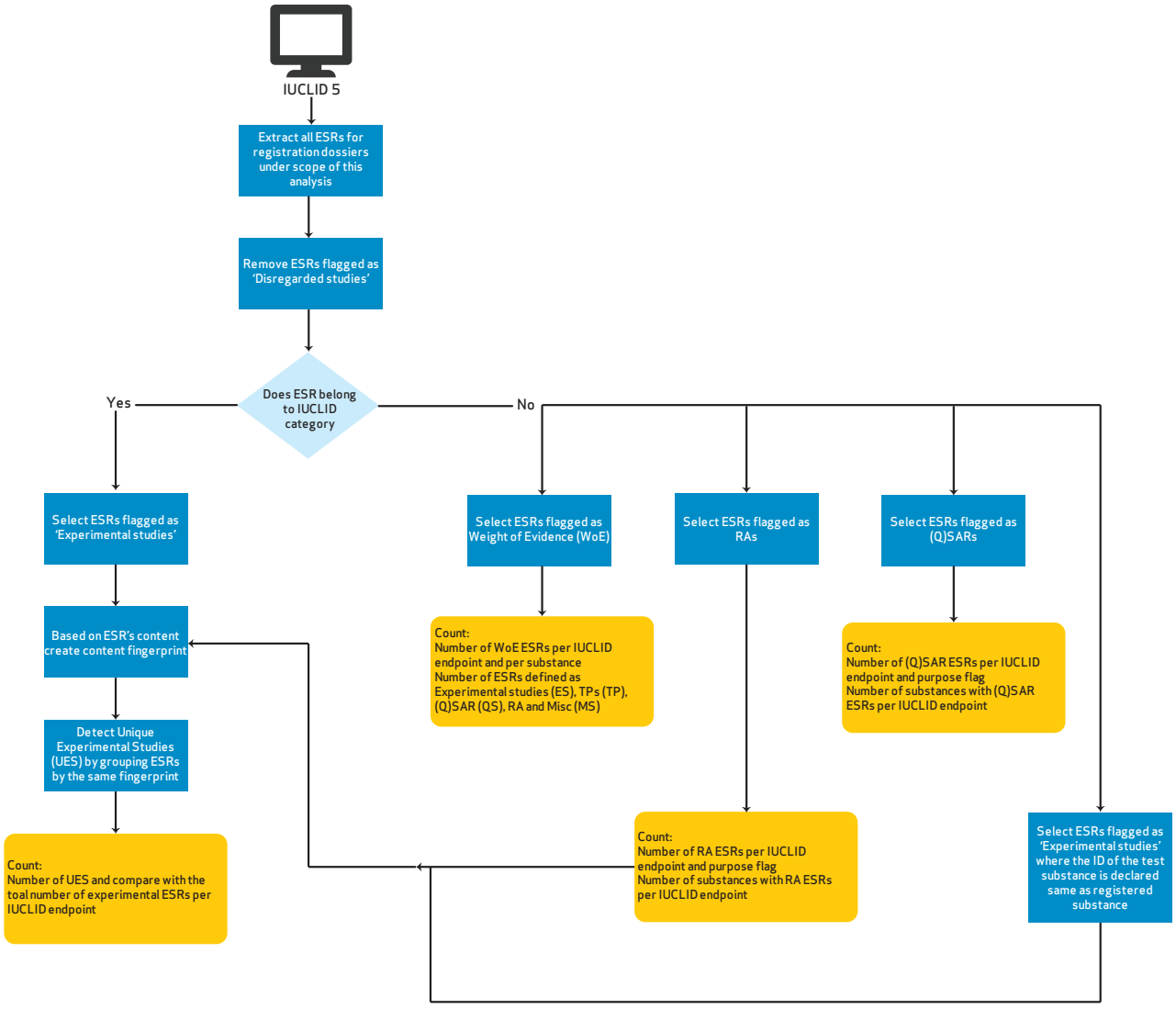


Figure 4: Data extraction workflow for the analysis of used adaptations of Annex XI of the REACH Regulation

category dossiers, where read-across was used. To do this, the UESs were also identified for all ESRs flagged as read-across in non-category dossiers and the ESR substitution ratio for read-across (without the template) was calculated based on the following formula:

$$\textit{ESR Substitution Ratio [RA]} = \frac{\#ESR_{[RA]} + \#ESR_{[Exp^*]}}{\#UES_{[nonCAT]}}$$

Where:

$\#ESR_{[RA]}$- Number of all ESRs flagged by registrant as read-across in non-CAT dossiers,

$\#ESR_{[Exp^*]}$- Number of ESRs flagged by registrant as experimental studies where the content fingerprint is identical to at least one read-across ESR in non-CAT dossiers.

$\#UES_{[nonCAT]}$- Unique experimental studies identified for all read- ESRs in non-CAT dossiers.

3.4.4 Main findings

Detailed results of these analyses are presented in Figures 12.1-12.4 and Tables 12.1-12.5 of Appendix 12. A number of points are highlighted in this section.

In terms of the use of more sophisticated analytical methods, it was found that on average the read-across approach was used more frequently than the weight of evidence approach, which in turn was used more often than (Q)SARs and other computational techniques, where registrants chose to define study result type as 'estimated by calculation' (see Figures 12.1-12.4 in Appendix 12). This is in line with the results of the substance-based approach presented in Section 3.4.

In non-category IUCLID template dossiers, the ESRs reported by registrants as being read-across information could be considered as being either a key study (39%), a supporting study (34%), part of a weight of evidence (23%) or not assigned (4%)

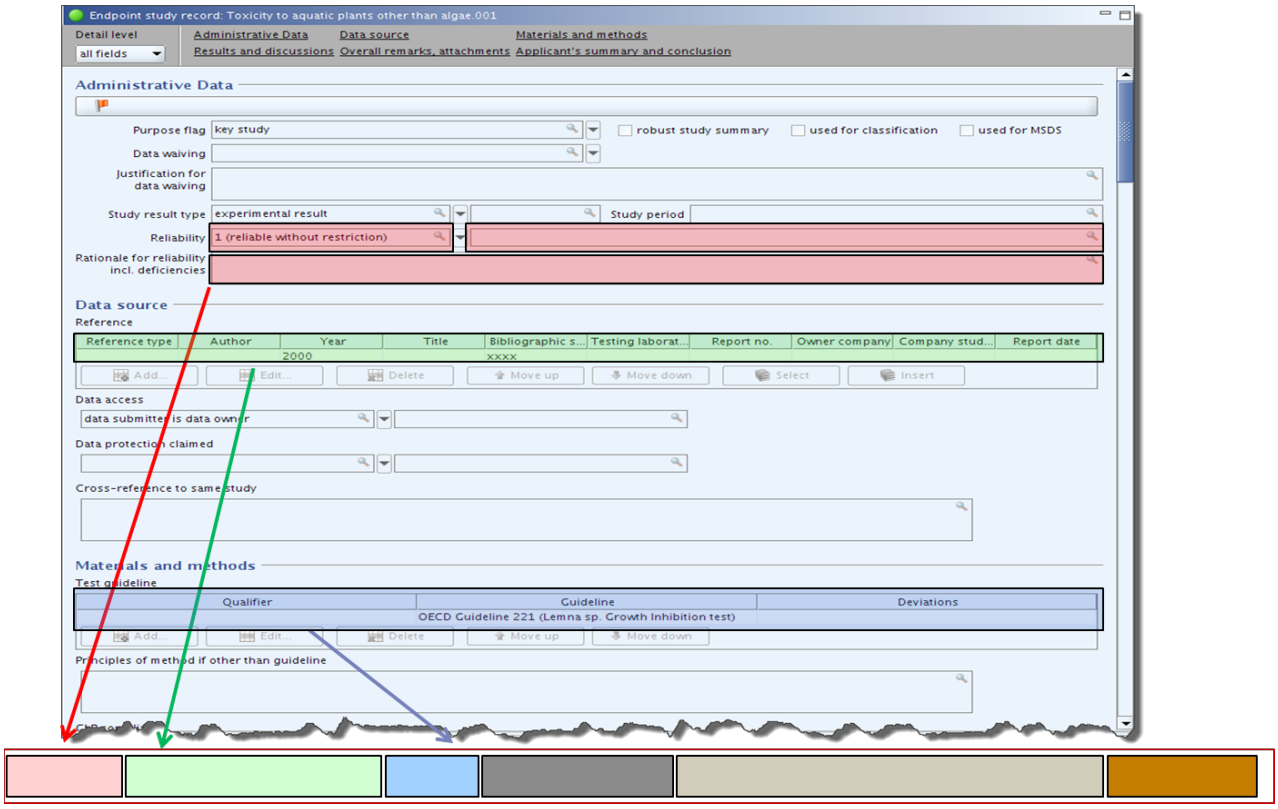


Figure 5: General schema of fingerprint generating for identification of the UES.

(Table 12.1, Appendix 12). In those cases where (Q)SAR/calculated results were reported in the ESR, on average they were regarded as the key study in 35% of cases, as a supporting study in 32% of cases and as part of a weight of evidence in 32% of cases (Table 12.3). In the weight of evidence cases that were identified, these mainly comprised the use of read-across (average 50% of the ESR) and experimental studies (41% of ESR) (Table 12.4). The use of these different adaptation possibilities are presented in Table 12.5, which shows that read-across is the predominantly used adaptation possibility.

The frequency of using read-across differs noticeably between endpoints. Analyses of the use of read-across in the standard registration dossiers and category dossiers, for which a special category template was used, showed that read-across was used across a range of endpoints. The differences were greatest in non-template category dossiers. In these cases, the greatest use of read-across (based on the number of substances) was for repeat dose toxicity, acute toxicity, toxicity to reproduction and developmental toxicity for the human health endpoints and short-term toxicity to fish for environmental endpoints. Read-across seems to be used less frequently for the REACH requirements on "long-term toxicity to birds" and "carcinogenicity". However, this might be explained by the fact that both endpoints are not standard information requirements under the REACH Regulation and the studies may not be always required unless triggered by adverse findings of the lower tier tests.

Furthermore, cases with the IUCLID category template appear to have a more extensive use of read-across than individual cases or cases that are recorded without the use of the IUCLID template. Many more of the available endpoint study records containing unique experimental data are found in registrations that do not use the IUCLID category template (see Table 12.2).

3.5 NEW TESTING PROPOSALS ON VERTEBRATE ANIMALS SUBMITTED BY THE SECOND REGISTRATION DEADLINE

As reported in the first report pursuant to Article 117(3) of the REACH Regulation, by February 2011 the Agency received testing proposals in 574 dossiers covering a total of 1 175 tests, of which 711 were vertebrate animal studies.

After the second registration deadline has passed, ECHA published the detailed registration statistics on it's website[14]. Companies submitted a total of 770 new testing proposals for phase-in substances produced at or above 100 tonnes per year in 376 dossiers. Additional analysis of all the dossiers available in the database used for analysis by 1 October 2013 (all tonnages bands, phase-in and non-phase-in substances, including late 2010 registrations) revealed that companies had submitted a total of 933 proposals in 461 dossiers, including the endpoints also covered by a read-across. New tests were also proposed in updated dossiers, incoming late registrations and for non-phase-in substances. Among those new testing proposals, in four cases they appeared to be inadmissible – either tests were already performed or on-going.

In total, 701 new tests on vertebrate animals were proposed. Of those, 563 were proposals to test on vertebrate animals to fulfil the REACH information requirements listed in Annex IX. The other tests were proposed either for late registrations of the highest tonnage band, or for the endpoints which do not require vertebrate animal testing (for example, tests for viscosity or long-term toxicity for aquatic intervertebrates). However, when analysing dossiers for the first report, ECHA did not take into account whether the test was proposed with target or source substance, while this distinction was performed for the testing proposals submitted by the 2013 registration deadline. In 56 cases where registrants proposed to test analogue substances, the number of actual tests to be performed will depend on whether ECHA and Member States conditionally accept the proposed approach.

14 http://echa.europa.eu/information-on-chemicals/registered-substances/reach-2013/registrations

Table 6: Testing proposals submitted to ECHA by 1 October 2013 (all tonnages, phase-in and non-phase-in substances, including IUCLID category dossiers)

Endpoint	Number including read-across	Number excluding read-across
Repeated dose toxicity (oral)	222	200
Repeated dose toxicity (dermal)	25	24
Repeated dose toxicity (inhalation)	1	1
Genetic toxicity (*in vivo*)	41	41
Toxicity to reproduction	72	65
Developmental toxicity	308	283
Bioaccumulation: aquatic / sediment	7	7
Long-term toxicity to fish	23	22
Total	701*	645*

*two tests were proposed for non-standard REACH information requirements: direct observations (clinical cases, poisoning incidents and other).

3.6 TESTS CONDUCTED IN 2009 OR LATER FOR EACH ENDPOINT REQUIRING VERTEBRATE/ INVERTEBRATE TESTING

To identify the number of new studies, using an IT-based data mining and analysis, the following working assumption was made: ECHA only takes into account the records of experimental studies with a reference date of 2009 or later, as these studies may have been conducted to fulfil the REACH requirements. If exact information on the study period was provided, a cut-off date of 1 June 2008 or later was used to give more accurate results.

This assumption has led to an overestimation rather than an underestimation of the number of cases for two reasons. Firstly, it is acknowledged that some of the studies may have been conducted for purposes other than REACH, and as REACH requires, registrants have obligations to provide all relevant studies that are available. Registrants do not have the obligation to include the reasons why studies were conducted in their registration dossiers. Consequently, new studies conducted for non-REACH purposes cannot reliably be identified using computerised searches. Secondly, only limited information is available on the dates a study was conducted. Registrants often only provide the date of the final study report. Depending on the type of study, it can take many months or years from initiation of a study until a final report is available. As such information may not be present in registrations, there are limitations to what can be achieved using data mining.

The basis for assigning a study as being "newly performed" or not was to take the oldest date from IUCLID dossier fields where date information was provided. For example, registrants may have indicated that the study started before 1 June 2008 even though the date of the final report was much later. Using the UES approach, duplicate records of the same study were filtered out from the numbers as presented in Table 7.

In the case of higher tier studies, a further screening of information extracted from registration dossiers was performed. This allowed cases to be distinguished where, for example, the registrants incorrectly reported information from *in vitro* studies as *in vivo* studies, or where dose range finding studies, conducted in preparation for other higher tier vertebrate tests, were also reported as new unique studies. All such cases (altogether 128 studies) were eliminated from the number of new higher tier vertebrate animal tests listed in Table 7[15].

15 This table covers 3 813 lead and individual dossiers covering 3 662 phase-in and non-phase in substances at or above 100 tonnes per year and 649 lead and individual category dossiers covering 523 substances at or above 100 tonnes per year

Endpoint name	Species Usually Tested	Annex X [#UES]	Annex IX [#UES]	Annex X [#UES] CAT	Annex IX [#UES] CAT	Total
Bioaccumulation aquatic/ sediment	*in vitro*	1	0	0	0	3
Skin corrosion/irritation	*in vitro*	227	443	8	4	682
Eye irritation	*in vitro*	90	255	15	3	363
Skin sensitisation	*in vitro*					54
Genetic Toxicity	*n vitro*	652	1222	28	50	1952
Total Number of 'new' experimental studies *in vitro*						3052
Bioaccumulation aquatic/ sediment	fish	10	6	0	0	16
Short-term toxicity to fish	fish	212	423	2	8	645
Long-term toxicity to fish	fish	34	31	1	0	66
Long-term toxicity to birds	bird	3	0	0	0	3
Acute Toxicity (Oral)	Rat or Mouse	151	304	3	6	464
Acute Toxicity (Inhalation)	Rat or Mouse	100	112	9	0	221
Acute Toxicity (Dermal)	Rat or Mouse	144	311	4	9	468
Skin corrosion/irritation	Rabbit	103	177	6	5	291
Eye irritation	Rabbit	176	291	13	5	485
Skin sensitisation	Mouse and Guinea pig					721
Carcinogenicity	Rat or Mouse	6	0	0	0	6
Combined Screening study	Rat or Mouse	220	614	14	25	873
Genetic Toxicity	Rat or Mouse	51	79	1	4	135
Prenatal developmental toxicity	Rat or Mouse	47	29	5	1	82
Repeated dose toxicity (28 days, dermal)	Rat or Mouse	10	5	0	0	15

Repeated dose toxicity (28 days, inhalation)	Rat or Mouse	30	23	1	1	55
Repeated dose toxicity (28 days, oral)	Rat or Mouse	60	164	3	3	230
Repeated dose toxicity (90 days, dermal)	Rat or Mouse	0	0	0	0	0
Repeated dose toxicity (90 days, inhalation)	Rat or Mouse	9	6	2	0	17
Repeated dose toxicity (90 days, oral)	Rat or Mouse	32	28	2	0	62
Repeated dose toxicity chronic (all routes)	Rat or Mouse	0	0	0	0	0
Toxicity to reproduction (other)	Rat or Mouse	2	1	0	0	3
Toxicity to reproduction (one generation)	Rat or Mouse	2	4	0	0	6
Toxicity to reproduction (two generation)	Rat or Mouse	11	8	4	0	23
Total Number of 'new' experimental studies *in vivo*						4887
Total Number of 'new' experimental studies						7939

The total number of "new" experimental studies identified after the 2013 registration deadline has almost increased twice when compared to the data published in 2011 (7 939 and 3 340 tests conducted, respectively). As shown in Table 7, and in line with the first report, around a quarter (24.6%) of the new studies have been conducted on **genetic toxicity *in vitro***. Again, in line with the findings described in the previous report, 91.5% of all new studies were submitted to fill in the data gaps for the Annex VII and VIII endpoints for which testing proposals were not required, namely **acute toxicity, eye irritation, skin corrosion/irritation, skin sensitisation, sub-acute repeated dose toxicity, repeated dose/ reproductive toxicity screening study and short-term toxicity on fish**.

Regarding the performance of new studies on vertebrate animals required for Annexes IX and X after REACH entered into force, the majority of new tests were carried out for **bioaccumulation in fish, repeated dose toxicity** (sub-chronic and chronic duration, all routes), **pre-natal developmental** toxicity, and **reproductive toxicity**. On the overall number of registration dossiers of all tonnage bands, and both phase-in and non-phase-in substances analysed, ECHA has identified 328 unique new studies conducted on vertebrate animals relevant to five higher tier human health related endpoints and 85 unique studies conducted to fulfil information requirements for three higher tier environmental endpoints. Hence, this number represents 5.2% of the total number of "new" experimental studies. It should also be noted that 76 of these studies had been covered by the first report.

As reported above, 1 153 new **acute toxicity** studies with the date of 2009 or later were identified in the current data pool. The new acute toxicity studies were performed via the oral route (464 ESRs), via the dermal route (468 ESRs) and via the inhalation route (221 ESRs). The total number of new unique studies conducted for the endpoints of **skin corrosion/ irritation** was 973 new experimental studies of which 682 (70%) were *in vitro* studies. Regarding **eye irritation**, there were 848 new experimental studies found, 43% of which were *in vitro* tests. Regarding **skin sensitisation**, in total 775 new experimental studies were found of which 54 (7%) were *in vitro* studies. An exhaustive analysis of all studies (including *in vitro* tests), conducted for the three abovementioned endpoints, is provided in Appendices 2, 3 and 4.

In 2011, 129 new **repeated dose toxicity** studies (summarising all routes and duration of exposure) were identified, while in the current data pool (dossiers submitted by 1 October 2013), there was a noticeable increase of those studies: 379 new tests with the date of 2009 or later were found. From them, 230 new tests were conducted for Annex IX dossiers and 149 for Annex X registrations.

In line with the previous report and to ensure consistency of the analysis, all **reproduction toxicity screening** studies dated 2009 or later (performed according to OECD Test Guideline 422 or 421, and/ or to the various equivalent US EPA guidelines) have been counted and presented separately as they can be used to fulfil information requirements for Annex VII core data under different endpoints (i.e. repeated dose toxicity and reproductive toxicity). Hence, counting them at the endpoint level could lead to double counting, therefore potentially overestimating the number of tests conducted.

In total, 873 new unique **combined screening** studies were identified in the current data pool (234 in 2011). More than 44% of them were reported under the endpoint of **toxicity to reproduction**, following **repeated dose toxicity** endpoint (30% of the tests). In addition, 23% of the **screening** studies were used by the registrants to cover information requirements for **pre-natal developmental toxicity** testing. The registrants used 39 **screening** studies when building their category dossiers.

After subtracting the **screening** studies from the total number of studies entered for **reproductive toxicity** (i.e. one and two-generation reproduction toxicity studies), it appeared that registrants submitted 32 new **reproductive toxicity** studies. From them, 19 were identified in the pool of the highest tonnage band dossiers, while 13 unique studies were used to cover standard information requirements for the Annex IX dossiers. Four of those 32 studies were used in category dossiers.

After subtracting the screening studies from the total number of studies entered for **pre-natal developmental** toxicity, 82 studies dated from 2009 or later were detected in the current database (52

in the pool of the highest tonnage band and 30 in the dossiers for substances produced between 100 and 1 000 tonnes per year). Of these 82 tests, six new studies were used in category dossiers.

In addition, as seen from the current data pool, registrants conducted 16 studies on **bioaccumulation (fish)** dated from 2009 or later. Registrants conducted 431 new **short-term toxicity to fish** studies. Annex IX dossiers reached 431, and 214 tests to cover Annex X information requirements (while in 2011, a total of 254 new studies were conducted for this endpoint). In addition, 66 new experimental studies on **long-term toxicity to fish** and only three studies on **long-term toxicity to birds** were dated from 2009 or later.

Follow up on cases where higher tier vertebrate animal tests that may have performed without an ECHA decision on a testing proposal

In 2011, ECHA reported that an IT-based search conducted for statistical purposes showed 107 higher tier studies in vertebrate animals, which appeared to have been conducted in the absence of a testing proposal or ECHA decision. This finding does not necessarily mean that obligations under REACH have not been followed. If new tests are available (e.g. conducted for non-REACH purposes), registrants are obliged by REACH to include them in their registrations. ECHA noted[16] that a number of the reported tests had been requested under previous EU legislation and advised registrants to include their reasons for the submission of new studies rather than a testing proposal. It is not ECHA's remit to request the missing information. Therefore, the remaining cases were referred to the Member State authorities for their consideration for any possible follow-up actions.

With regard to the current analysis of new higher-tier studies (relevant to Annexes IX and X), ECHA identified that 293 studies appeared to have been conducted in the absence of an ECHA decision, whereas 44 tests were generated after receipt of an ECHA decision (under testing proposal examination or compliance check).

Some registrants provided reasoning as to why new tests were included in their dossiers. Examples for justifications checked by ECHA include that testing was triggered by non-EU legislation, or testing required by an MSCA decision for notified or existing substances (Dangerous Substances Directive 67/548 EEC, and Existing Substances Regulation 793/93 EEC). When ECHA observes that a test was performed for which an ECHA decision is required under REACH, the relevant Member States will be informed and will be responsible for taking enforcement actions where appropriate.

In 14 cases, a testing proposal had been submitted but ECHA has not completed the examination process as it was noted that testing was already ongoing or done. In these cases, ECHA has already communicated the relevant details to the Member State authorities.

As noted previously, this number is likely to be an overestimation of the true number of cases. A screening of information extracted from the IUCLID dossiers indicated that the new test was acquired from another entity (72 cases) or that the testing was generated to meet other regulatory purposes (39 cases). In the remaining 167 cases, the computerised search did not ascertain whether the registrant has included reason for the submission of these new studies. The Member State authorities have been informed of the details of these cases for their consideration of any further actions.

16 http://echa.europa.eu/documents/10162/13628/evaluation_report_en.pdf

4. ECHA's commitment to support registrants

This section summarises the continuous progress on ECHA's commitment both to promote the use of alternatives and to support registrants to comply with their duties under REACH. ECHA considers this overview of possible supporting actions beneficial, in particular, for the companies manufacturing or importing chemical substances from 1 to 100 tonnes per year and that are preparing their registration dossiers for the last deadline.

4.1 SCIENTIFIC PRIORITIES

ECHA is a regulatory organisation with a mission in a scientific and technical context. Scientific knowledge related to chemicals management is progressing on all fronts. Significant and rapid development is being made, especially in (eco)toxicology, with an emphasis on better understanding the biological mechanisms leading to an adverse effect, rather than just observing the effect. Systems biology, bioinformatics, increased understanding of modes of action and adverse outcome pathways will also affect the way chemicals are tested, or how their properties can be predicted, thus enabling reduction in traditional animal testing.

There is a wide range of properties assessment for chemicals: 'traditional' toxicology studies, *in vitro* tests, 'read-across'/'chemical categories', quantitative structure activity relationships ((Q) SARs) and 'high throughput screening' approaches. Research is needed to combine these approaches, perhaps into Integrated Testing Strategies (ITSs) and/or 'batteries of tests' e.g. Integrated Approaches to Testing and Assessment (IATA). In addition, to support such combined approaches, further fundamental research will be necessary into the biological mechanisms that underpin toxicity or ecotoxicity.

Other examples of scientific developments include effects on endocrine systems of humans and wildlife, hazards and risks posed by nanomaterials, and combination effects of chemicals.

These areas are seen as priorities for ECHA where the Agency needs to be fully aware of these developments when making judgments about the scientific adequacy of information provided by companies, when issuing regulatory opinions and decisions, or when providing guidance about how to fulfil the requirements of the legislation.

4.2 QSAR TOOLBOX PROJECT

One of ECHA's strategic aims is obtaining high quality information for safe manufacture and use of substances through registration. In this context,

ECHA investigates how the OECD QSAR Toolbox, a software developed for grouping chemicals and filling data gaps, could help. In particular, a number of improvements in the Toolbox are planned in ECHA's multi-annual work programme that intend to facilitate the use of the software for low tonnage industrial substances and therefore may be useful for registrants falling under the third registration deadline.

The OECD QSAR Toolbox is a software tool (freely available[17]) that has been developed by the OECD in cooperation with ECHA to support the grouping of substances. It contains different databases, pre-coded knowledge and statistical tools to allow grouping of substances and the elaboration of small local models for a particular chemical of interest. The Toolbox approach offers a flexible methodology for grouping of substances and potential filling of data gaps but, as with all other adaptations, needs proper description and documentation when utilised. The individual tools in the Toolbox should be understood well in order to ensure optimal use and credible results.

There is a lot of training material available from the website cited above. A valuable source of knowledge is the endpoint-specific training materials. The new capabilities of the OECD QSAR Toolbox have been described in several step-by-step examples on how to address data-gap-filling for acute aquatic toxicity and genotoxicity. An attempt is also made to illustrate the prediction of more complex endpoints. New capabilities are available in the Toolbox Version 3 that can estimate the toxicity of user-defined mixtures (note that this term does not reflect any specific regulatory definition but a mixture of chemicals with known composition; the composition is needed as an input for the tool), and allows tautomeric multiplication (this is important to consider if there is a tautomer that might be more reactive than the structure used as input).

There is not a unique way in which the Toolbox can be used. The flexibility of the tool leaves it up to the user to make cases and to assess their validity and applicability in a given context. A good practice for grouping is gradually reducing the number of analogues for the query substance, first by chemical and then by mechanistic similarity. The selected analogues should be associated with experimental data to be used as source substances for read-across or trend analysis.

According to a recent online study on the usability and usefulness of the OECD QSAR Toolbox[18] with more than 170 respondents, there is a general satisfaction with user-friendliness of the tool. The respondents indicated that read-across, profiling, data gathering, (Q)SAR applications, and identifications of analogues are the most frequent uses. Numerous suggestions for further development and improvement were also collected.

The OECD QSAR Toolbox will be further developed and improved before the third REACH registration deadline in 2018. The approaching development will focus on improving the usability and streamlining the workflows, improving the contents, increasing knowledge on the reliability of the individual components, and adding new functionalities. Fundamental for the improvement of the Toolbox will be to develop the way in which adverse pathway outcomes could be handled. This is in line with the OECD focus on this methodology in the next 5-10 years.

ECHA has organised a Toolbox training for its staff every year since its establishment in 2007. Two workshops with industrial users of the tool were organised in 2011 and 2012. In 2014, ECHA is planning to organise a training open also to experts from EU Member State competent authorities (MSCAs) and ECHA Committee members.

Last, but not least, in March 2014, ECHA published an illustrative example with the OECD QSAR Toolbox workflows: introductory note and case studies[19].

4.3 GUIDANCE ON INFORMATION REQUIREMENTS AND CHEMICAL SAFETY ASSESSMENT

Between June 2011 (when the first report pursuant to the Article 117(3) of the REACH Regulation was published) and June 2014, ECHA continued to develop REACH Guidance on Information

17 http://www.(Q)SARtoolbox.org

18 http://echa.europa.eu/en/view-article/-/journal_content/title/e-news-2-october-2013

19 http://echa.europa.eu/support/oecd-(Q)SAR-toolbox

Requirements and Chemical Safety Assessment (IR&CSA Guidance). The most up-to-date information on updates of this (and other) Guidance documents is available on the ECHA website[20].

With regard to the scope of this report, ECHA finds it noteworthy to highlight one of these updates. A draft updated Chapter R.7a (Sections R.7.7.1 to R.7.7.7 related mutagenicity only) was sent for comments to the Partner Expert Group in May 2013 and to the Member State Committee (MSC) and the Committee for Risk Assessment (RAC) in February 2014[21].

This update primarily takes account of the adoption in July 2011 of an OECD test guideline (OECD TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays[22]). Further to this, an update of the text to reflect the status of development of some of the test methods mentioned in those sections, as well as a range of editorial corrections and minor amendments are proposed. In particular, the adoption of the OECD TG 487[23] for the *in vitro* Micronucleus test is now mentioned in the guidance and the subsection on Non-testing data on mutagenicity now contains details on the OECD QSAR Toolbox.

4.4 PRACTICAL GUIDES

In 2011-2014, ECHA also continued to update practical guides[24].

In line with the scope of this report, ECHA notes that Practical Guides 1 (How to report *in vitro* data) and 3 (How to report robust study summaries) were updated in September and November 2012, respectively. Revision of the Practical Guide 3 addressed structure and content in relation to the updated sub-chapter R.7.1 'Physicochemical properties' within the 'Guidance on information requirements and chemical safety assessment R.7a: Endpoint specific guidance' and new or revised OECD Test Guidelines.

Practical Guides 5 (How to report (Q)SAR) and 6 (How to report read-across and categories) were updated in September 2013 without major/substantial changes (editorial changes, link refresh, introducing updates on existing topics, e.g. update of the last version of the Toolbox cited).

4.5 SPECIALISED WORKSHOPS AND WEBINARS

In addition to the more general interest conferences and events, ECHA organises expert workshops for specialised audiences to gain insight and feedback from industry on specific areas. These have included workshops on the development needs of the OECD QSAR Toolbox, particularly in relation to additional support in view of the registration deadline in 2013 and an expert workshop on read-across assessment in partnership with ECHA's industry stakeholders.

The read-across workshop organised at ECHA with active support from Cefic-LRI on 2-3 October 2012 focused on exchanging views on evaluation of read-across arguments in a dossier between ECHA, the Commission and Member States and to expand the discussion with stakeholders on what constitutes a robust scientific justification for read-across. Over 100 participants from ECHA, Member States, the European Commission, OECD, academia, non-governmental organisations and industry took place at this workshop. The workshop documents and report are available on ECHA's website[25].

On 4 October 2012, ECHA also hosted an expert scientific discussion group on the adequacy of two *in vivo* tests where ECHA invited experts with regulatory, scientific, industrial and stakeholder backgrounds for an open discussion on the use of the transgenic rodent gene mutation assay

20 http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

21 http://echa.europa.eu/support/guidance-on-reach-and-clp-implementation/consultation-procedure

22 http://www.oecd-ilibrary.org/environment/test-no-488-transgenic-rodent-somatic-and-germ-cell-gene-mutation-assays_9789264122819-en;jsessionid=380vu3hdkhri5.x-oecd-live-01

23 http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en

24 http://echa.europa.eu/practical-guides

25 http://echa.europa.eu/en/view-article/-/journal_content/title/experts-workshop-on-read-across-assessment-with-active-support-from-cefic-lri

(TGR, OECD TG 488[26]) and the unscheduled DNA synthesis assay (UDS, OECD TG 486[27], B.39 Test Methods Regulation) under REACH. The aim of the meeting was to produce a report about the scientific adequacy of both assays to support stakeholders in their testing strategy decisions. 44 experts from 16 Member States or associated Member State competent authorities, the European Commission, the European Medicines Agency, the European Food Safety Authority, industry, consultants, contract research organisations and non-governmental organisations participated in a technical discussion. The conclusions of the discussion are published on ECHA's website[28].

Several virtual events[29] have been organised by ECHA on information requirements including topics such as read-across, weight of evidence, *in vitro* data and (Q)SARs. ECHA has hosted four webinars for lead registrants on information requirements that were attended by 476 participants. A total of 87 questions were submitted through the webinar question and answer tool. Since 2012, ECHA has also organised a webinar series entitled "How to bring your registration dossier in compliance with REACH" that provides detailed information to registrants on how they can improve the quality of their dossiers. A total of 751 participants attended the webinar series and nearly 120 questions were answered by the panellists.

In February 2013, ECHA hosted an EPAA-Cefic organised training workshop on "*Skin sensitisation - Moving forward with non-animal testing strategies*". The aim of the workshop was to bring industry and regulators together and to discuss the use of alternative test methods that are currently under validation for the endpoint of skin sensitisation. More information about the workshop can be found from the published flash report of the workshop[30] and from a peer-reviewed publication (Skin sensitisation - moving forward with non-animal testing strategies for regulatory purposes in the EU (2013), Basketter et al., Regul Toxicol Pharmacol.).

As a part of its campaign for the second REACH registration deadline in 2013, ECHA provided specific support to small and medium-sized companies (SMEs). The Agency organised several workshops and a webinar in particular for SMEs. In 2012, ECHA organised two workshops for lead registrants, and reimbursed SME lead registrants wishing to attend the event in an effort to make sure that SME lead registrants had the possibility to learn from other lead registrants preparing for the 2013 REACH deadline. The one-to-one and training sessions organised between event participants and ECHA staff for the Agency's flagship events such as the Lead Registrant Workshops and Stakeholders' Day conferences also give the possibility for SMEs to ask specific questions related to their obligations and to gain practical experience in preparing their registration dossiers. ECHA's reimbursement practice for meetings and events also ensures that accredited stakeholder organisations with a majority of SME companies as their members, are reimbursed by ECHA.

ECHA also organised a webinar in June 2012, covering the SME verification process undertaken to identify SMEs and an overview of costs faced by them in the REACH registration phase. The webinar was attended by nearly 600 participants.

The Agency co-operates with the Enterprise Europe Network, which supports small companies through local chambers of commerce, technology centres, research institutes and development agencies. In November 2013, ECHA organised training for the Network partners to learn more about REACH and its impact on their clients. The training included dedicated information about SMEs, promoted participation in ECHA public consultations and detailed the available support. The Agency aspires to act as a catalyst for local cooperation between national helpdesks and Enterprise Europe Network partners.

26 http://www.oecd-ilibrary.org/environment/test-no-488-transgenic-rodent-somatic-and-germ-cell-gene-mutation-assays_9789264203907-en;jsessionid=axsgi19ie2kt.x-oecd-live-01

27 http://www.oecd-ilibrary.org/environment/test-no-486-unscheduled-dna-synthesis-uds-test-with-mammalian-liver-cells-in-vivo_9789264071520-en

28 http://echa.europa.eu/en/view-article/-/journal_content/title/scientific-discussion-on-the-adequacy-of-two-in-vivo-tests

29 http://echa.europa.eu/support/training-material/webinars

30 http://www.cefic-lri.org/uploads/Events%202013/skinsens%20RT%20flash%20report.pdf

A workshop for SMEs was organised jointly with the European Commission in December 2013 and aimed to find solutions for challenges faced by SMEs preparing for REACH.

4.6 PUBLICATION OF ADOPTED ECHA EVALUATION DECISIONS

From December 2012, ECHA started publishing non-confidential versions of all dossier evaluation decisions originating from compliance checks and the examination of testing proposals on the Agency's website. These decisions are available and in most cases with a link to the related aggregated registration information as contained in ECHA's dissemination portal.

ECHA notes that the published documents represent decisions with blanked out sections that have been claimed confidential by the registrant and were deemed to harm their commercial interest if disclosed. In addition, any personal data is removed from the documents. The decisions are only available in their original language.

By publication of the dossier evaluation decisions, ECHA further increases its transparency and offers registrants and third parties a new opportunity to follow and increase their insight into the outcome of the evaluation processes of compliance check and testing proposal examinations. For instance, the published decisions on testing proposals for the different categories and read-across give a good insight in important elements for a potentially successful approach. Registrants who recently submitted or are still preparing their dossiers, could still use the opportunity to review their existing data, and proactively update them if they gain important learnings from a review of these decisions. Interested third parties and non-governmental organisations who can send scientifically-relevant information during third party consultations on proposed tests, may benefit from the overview on how ECHA and the Member States dealt with such information and what are the areas of improvement (see Section 2.6 of this report also).

By 1 March 2014, ECHA has issued more than 650 dossier evaluation decisions and out of them more than 400 have been published[31]. The number of decisions published in a monthly batch varies due to the unpredictable outcome of the consultations and the cases being in different phases of the consultation process.

In 2013, the first substance evaluation decisions have been taken. By 1 March 2014, the Member State Committee agreed that ECHA should request more information on 22 substances because there is currently an insufficient amount of information to enable an assessment on the risks that they pose to human health and/or the environment. More information on the scope of these decisions is outlined in the minutes of the respective MSC meetings[32] and briefly presented in the 2013 evaluation report[33]. The public versions of the first agreed substance evaluation decisions will become available shortly on ECHA's website.

31 Moving number, most recent update available at: http://echa.europa.eu/regulations/reach/evaluation/requests-for-further-information/evaluation-decisions

32 http://echa.europa.eu/about-us/who-we-are/member-state-committee/meetings-of-the-member-state-committee

33 http://echa.europa.eu/regulations/reach/evaluation

5. Summary and conclusions

The principle in REACH of the sharing and joint submission of hazard information on a substance continues to work well in general, as also concluded for the dossiers submitted for the first registration deadline. The registrants used this principle to fulfil the information requirements and to avoid unnecessary animal testing.

Based on the analysis of Annex IX dossiers in 2010 and 2013, the overall picture is that, generally speaking, fewer experimental studies and more alternatives were available across the majority of endpoints in the more recent registrations. Given that the standard information requirements are the same in 2010 and 2013 for these Annex IX registrations, these data tend to show that registrants for the 2013 registration deadline appeared to have less existing tests available and made greater use of alternatives such as read-across and weight of evidence.

Taking all of the different data mining analyses together, the consistent finding is that use of read-across is the key alternative approach found in the registration dossiers. In particular, this approach is used for the higher tier endpoints where alternative non-animal test methods and testing strategies approved for regulatory use are not yet available. Regarding the most widely used alternative approach – read-across – ECHA has reminded registrants that it is their responsibility to build their cases appropriately. ECHA has already conditionally accepted several big categories of substances in the context of testing proposal examinations.

The endpoints for which most testing proposals are presented by registrants are repeated dose toxicity and developmental toxicity. This observation appears to be consistent with the ESR analysis conducted for this report, which showed that existing experimental data for these endpoints is less frequently available in registrations in Annex IX registrations in 2013 compared to that seen in 2010.

Detailed analysis for the skin corrosion/irritation endpoint based on the data provided for the 2013 registration deadline clearly shows that alternative means to fulfil the information requirements for the skin corrosion/irritation endpoint is used. The registrants have made use of alternative approaches e.g. in many cases the information requirement was fulfilled by solely using *in vitro* test data. The registrants have also made use of information obtained by applying read-across strategies (analogue and category approaches) to avoid unnecessary animal testing.

Data analysis for the eye irritation endpoint showed that the registrants are using *in vitro* test methods in their assessment of eye irritation potential, even

though the currently available *in vitro* test methods do not have a potential to fully assess this hazard for all substances. In cases where the results from the *in vitro* test methods do not allow a conclusion to be drawn on the classification of the substance, the registrants have performed an *in vivo* study. ECHA notes that this approach is in line with current practice described in the ITS approaches of the ECHA guidance documents and OECD test guideline appendices.

Some registrants have already made use of a test battery for the skin sensitisation endpoint. When comparing the use of *in chemico/in vitro* studies in the dossiers analysed for the current and for the previous report, there is a clear tendency for the alternative methods for this endpoint to be used more often, even though this approach is still in its early stage.

The third party consultation process on testing proposals is working in that third parties (mainly NGOs concerned with animal welfare and companies with an interest in the substance) frequently send comments of a scientific nature on testing proposals, which are published on ECHA's website. In the past, ECHA has highlighted how third party comments might be improved so that registrants may better understand how the approaches might be used to fulfil the information requirements where this is possible. It has become more typical that third parties often provide scientifically-based considerations and information on the use of alternatives (e.g. approaches based on the use of read-across with or without weight of evidence). In a number of cases, registrants appeared to have used the information provided to remove the testing proposals by either submitting an adaptation to the information gap or, rarely, by including actual data on the substance itself. ECHA has addressed the outcome of these consultations in the annual evaluation progress reports.

This report also provides the number of new studies that appear to have been conducted for the purpose of the REACH Regulation. Such new studies were again performed largely for the core Annex VII and VIII data obligatory for registration, as would be expected, because higher-tier Annex IX and X studies require the approval of testing proposals beforehand. To fill data gaps for the higher tier information gaps, registrants included 701 testing proposals for vertebrate animal studies in dossiers submitted by the 2013 registration deadline. From those, 563 were proposals to test on animals to fulfil the REACH information requirements listed in Annex IX. For 56 of these (so-called) read-across testing proposals, registrants intend to use the outcomes from testing of other substances.

ECHA stresses again that the reports that are provided to the Commission pursuant to Article 117(3) of the REACH Regulation do not address the quality of information in the registration dossiers. Nor do they address compliance of the registrations with the provisions of the REACH Regulation. However, from its further compliance check dossier evaluation work, ECHA has noted that the adaptations used by the registrants are often inadequately justified and contain deficiencies. In these cases, ECHA will issue a decision requesting missing information. This means that more animal tests may be necessary.

Finally, ECHA will continue in its efforts to promote the use of alternatives through its publications, website, guidance development, campaigns, events and through the dissemination website. In particular, ECHA will continue to promote the correct use of read-across, and is developing its framework to guide the consistent assessment of read-across and grouping approaches as presented in registration dossiers. Lessons learnt from this exercise will be used to develop case examples for its website and guidance documents, as well as further advice to registrants and stakeholders.

Appendices

Appendix 1: Acute toxicity

As already addressed in the previous report, the information requirement for an acute toxicity study conducted using the oral route applies at or above one tonne per year (Annex VII) and is therefore part of the core data for all the registrations. The requirement for such a study can be adapted, for example, if the substance is corrosive.

Acute toxicity by either dermal or inhalation exposure, or in some cases both routes, is needed for all substances (except gases) at or above 10 tonnes per year (Annex VIII), depending on the likely human exposure, and is therefore also part of the core data for the registrations in this study. The purpose is to have information on the toxicity of a chemical substance. The standard laboratory animal species used for this purpose is the rat, but the mouse is also used. The effects of the administered dose(s) are monitored and reported according to EU TMR/OECD TG standard protocols ensuring that the results can be used worldwide.

One *in vitro* test method, the 3T3 Neutral Red Uptake cytotoxicity assay, has been validated for identifying unclassified (acute oral toxicity, ≥2 000 mg/kg body weight) chemicals. However, the recommendation is to use it, for example, in an Integrated Testing Strategy (ITS) together with other methods, and not as a standalone method[1].

1 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/files-3t3/ReqNo_JRC79556_lbna25946enn.pdf

Available alternative approaches are therefore still mainly prediction methods (read-across and grouping) or weight of evidence using experimental methods in combination with prediction methods. In analysing this endpoint, records were observed addressing all three routes of administration (oral, dermal, or inhalation).

As presented in Figure 1.1, for dossiers for phase-in substances between 100 and 1 000 tonnes per year, 10 854 entries (100%) have been submitted. Experimental studies have been used for 4 265 (42.6%) of the ESRs. The registrants have used read-across in 3 009 (27.7%) of these entries, followed by the choice to omit the study (1 762 (16.2%)).

A weight of evidence approach has been used in 1 265 (11.7%) of all ESRs and other information sources for covering this endpoint have been used in 143 (1.3%) of the ESRs. In 50 cases (0.5%), (Q)SAR was used as the ESR.

The percentages of different ESR types for phase-in substances at or above the 1 000 tonnes per year range did not vary significantly from those of the phase-in substances between 100 and 1 000 tonnes per year, with the exception of a higher percentage of experimental studies (58.5%) and a lower number of proposed read-across cases (20.8%).

For the non-phase-in substances at or above 100 tonnes per year that were analysed in 2013, the total percentage of entries for experimental studies was also higher than in those analysed in 2011 (50.1% and 38.9%, respectively). Detailed results including relative differences between both data pools are provided in Table 3, of the main text.

Acute toxicity - all (HH)		
	No. ESR	% ESR
ES	4 625	42.6
TP	0	0.0
RA	3 009	27.7
FO	1 762	16.2
WE	1 265	11.7
QS	50	0.5
MS	143	1.3
Total	10 854	100

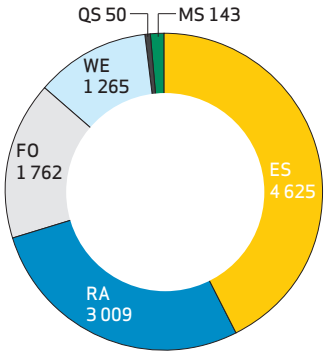


Figure 1.1: Acute Toxicity (all routes, 1 882 dossiers covering phase-in substances 100 - 1 000 tonnes per year, one or more ESR may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 2: Skin irritation/corrosion

The studies used to investigate irritation and/or corrosion predict the local effects of the test substance on humans at the site of first contact (skin, eye, mucous membrane of respiratory or gastrointestinal tract) after a single exposure. Observed local effects can be further differentiated as either irritant or corrosive effects, depending on their severity, reversibility or irreversibility. For *in vivo* studies, the substance to be tested is applied in a single dose to the skin of an experimental animal for four hours; the preferred species being the albino rabbit. Untreated skin areas of the test animal serve as the control.

The standard information requirements for this endpoint are provided in Annexes VII and VIII of the REACH Regulation and differ depending on the tonnage band. Annex VII (1 to 10 tonnes per year) requires only *in vitro* studies, while Annex VIII (10 – 100 tonnes per year) requires a confirmatory additional *in vivo* test, unless it is possible to conclude on the substance classification by using alternative options or the substance is classified as an irritant or corrosive.

Alternative options to fulfil standard information requirements for this endpoint under REACH include prediction methods, a weight of evidence approach and possibilities to adapt information requirements according to column 2 of Annexes VII and VIII. The potential to cause irritation or corrosion can also be predicted based on physicochemical properties of the chemical (for example, the substance is a strong acid/base or is spontaneously flammable).

According to Annex XI 1.4, registrants can also adapt the standard information requirements for an *in vivo* study based on the results of *in vitro* studies. Since a single *in vitro* study only addresses either skin corrosion or skin irritation potential, a tiered testing strategy may be needed to address the whole endpoint depending on the outcome of the first study performed.

There are validated *in vitro* methods available for this endpoint that can be used by registrants in a tiered testing strategy e.g. within a weight of evidence approach to fully replace testing on animals. For studying skin corrosion/severe irritation, these methods include, for example, the EU Test Method Regulation (TMR)/OECD TG standard protocols such as the transcutaneous electrical resistance (TER) test, the human skin model test (based on reconstructed human epidermis) and the membrane barrier test. For skin irritation, reconstructed human epidermis test methods are available.

In Table 3 of the main text, which shows the ESRs

for skin irritation/corrosion, the total number of entries for this endpoint separated by *in vitro* tests is summarised.

As presented in Figure 2.1, for dossiers for phase-in substances between 100 – 1 000 tonnes per year, 684 (100%) entries have been counted for *in vitro* studies for the endpoint in total. Experimental study flags have been used for 479 (70%) of all ESRs for this endpoint. A read-across approach is the second most commonly used approach for the *in vitro* skin irritation/corrosion endpoint where it was flagged by registrants in 118 (17.3%) cases. In 82 (12.0%) of the entries, a weight of evidence flag was used. Three of the registrants have used IUCLID flags to omit the *in vitro* study.

When comparing the *in vitro* entries for phase-in substances between 100 – 1 000 tonnes per year with the previous report, the total number of entries submitted has increased substantially (24 *in vitro* ESRs submitted by the 2010 registration deadline). Comparison of the distribution of the options to fulfil information requirements with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 3 of the main text) resulted in a relative decrease of 13.3% for experimental studies and a relative increase in the use of read-across (5.9%) and weight of evidence (9.8%).

In contrast with the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of *in vitro* ESRs was around 25% higher (443 vs 325 ESR entries). Comparison of the distribution of options to fulfil information requirements for *in vitro* skin irritation/corrosion among the ESRs with the results for dossiers at or above 1 000 tonnes per year described above (see Table 3 of the main text) did not result in significant differences.

For the non-phase-in substances produced at or above 100 tonnes per year, 35 *in vitro* skin irritation/corrosion ESRs were submitted in total (in the previous report only one entry was provided). For these substances, the registrants have mainly used the option experimental study flag (82.9%) and read-across approaches (14.3%).

In Table 3 of the main text, which shows the ESRs for skin irritation/corrosion, the total number of entries for this endpoint separated by *in vivo* tests is summarised.

Skin irritation/corrosion – *in vitro* (HH)		
	No. ESR	% ESR
ES	479	70.0
TP	0	0.0
RA	118	17.3
FO	3	0.4
WE	82	12.0
QS	0	0.0
MS	2	0.3
Total	684	100

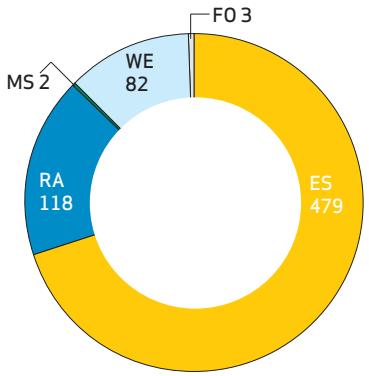


Figure 2.1: Skin irritation/corrosion *in vitro* (1 870 dossiers covering phase-in substances 100 – 1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

As presented in Figure 2.2, for dossiers for phase-in substances between 100 – 1 000 tonnes per year, 4 198 (100%) entries have been counted for *in vivo* studies for the endpoint in total. Experimental studies have been used for 2 035 (48.5%) of all ESRs for this endpoint. A read-across approach is the second most commonly used approach for the *in vivo* skin irritation/corrosion endpoint where it was proposed by registrants in 1 163 (27.7%) cases. In 630 (15.0%) of the entries, a weight of evidence approach was used. In 302 (7.2%) of the total entries, the registrants have proposed to omit the *in vivo* study. (Q)SARs were used in 47 (1.1%) and other information has been provided in 21 (0.5%) cases.

When comparing the *in vivo* entries for phase-in substances between 100 – 1 000 tonnes per year with the previous report, the total number of submitted *in vivo* entries has increased by 3 598 ESRs. Comparison of the distribution of the options to fulfil information requirements with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 3 of the main text) resulted in a relative decrease of 18.5% in the use of experimental study flags and relative increases in read-across (5.9%) and weight of evidence (9.8%) approaches.

In contrast with the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of *in vivo* ESRs was around 30% higher (6 676 vs 5 216 ESR entries). Comparison of the distribution of options to fulfil information requirements for *in vivo* skin irritation/corrosion among ESRs with the results for dossiers at or above 1 000 tonnes per year as described above (see Table 3 of the main text) did not result in significant differences.

For the non-phase-in substances produced at or above 100 tonnes per year, a total of 347 *in vivo* skin irritation/corrosion ESRs were submitted which shows an increase of over 100% in the total number of ESRs submitted compared to the previous report (see Table 3 of the main text). Comparison of the distribution of options to fulfil information requirements showed relative increases in experimental studies (14.1%), weight of evidence (7.6%) and read-across (4.1%) approaches. The use of other information and proposals to omit the study showed relative decreases (22.9% and 2.9%, respectively).

Skin irritation/corrosion – *in vivo* (HH)		
	No. ESR	% ESR
ES	2 035	48.5
TP	0	0.0
RA	1 163	27.7
FO	302	7.2
WE	630	15.0
QS	47	1.1
MS	21	0.5
Total	4 198	100

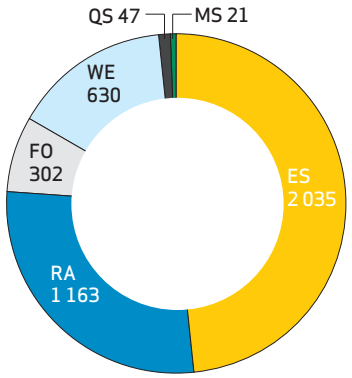


Figure 2.2: Skin irritation/corrosion *in vivo* (1 870 dossiers covering phase-in substances 100 – 1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Substance approach

A more detailed analysis has been performed for this endpoint due to the developments and international acceptance of alternative test guidelines. For this endpoint, the assessment has concentrated more on the substance level to obtain an understanding of how the registrants have used alternative test methods to fulfil the standard information requirements for their substance. For this substance level analysis, 2010 was chosen as the cut off year to identify new *in vivo* studies. The reason for deviating from the cut off year of 2009 specified in section 5.6 of this report, is because the *in vitro* skin irritation test methods were only adopted in July 2009 by the European Test Method Regulation. Therefore, for this particular analysis, a cut off year of 2010 was used to allow the registrants to take these new developments into account and to assess how registrants have made use of these new *in vitro* test methods.

In total, 3 807 dossiers[2] were analysed for this endpoint (1 862 dossiers in the previous report). Table 3 of the main text provides information on the types of strategies that the registrants have chosen to fulfil their standard information requirements for the skin irritation/corrosion endpoint. In total, 1 184[3] *in vitro* endpoint study records (ESRs) were submitted by the 2013 registration deadline (by the previous registration deadline, 354 *in vitro* ESRs were submitted). The number of the ESRs provided by the second registration deadline has increased by more than 100%. This shows that registrants are using alternatives to animal testing much more than they did for the 2010 registration deadline.

For 316 dossiers, the information requirement was fulfilled solely by using *in vitro* methods (8.5% of total dossier submissions). *In vitro* methods were also extensively used together with old *in vivo* studies (213 dossiers and 5.5% of total dossier submissions), as supporting information to read-across approaches (111 dossiers and 3.0% of total dossier submissions). This shows that the registrants have understood the value of *in vitro* studies to provide supporting evidence when only old *in vivo* data is available and when a read-across approach is followed.

In a few of the cases (2.5% of total dossier submissions), new *in vivo* studies were performed together with *in vitro* studies. These cases have not been fully evaluated, since they fall out of the scope of this report, however in a few of these cases, it seems that the *in vitro* and *in vivo* studies resulted in inconsistent results, and in some cases, *in vitro* studies were negative (i.e. non-irritant according to CLP) which were then followed by an *in vivo* study. The reason for an *in vivo* study resulting in a negative result from the *in vitro* skin irritation study, may be the need to investigate whether the substance needs a classification in line with optional category 3 according to the UN GHS (for example, if the registrant has responsibilities outside the EU), which the existing *in vitro* skin irritation study cannot address.

The registrants have also made use of read-across approaches (category or analogue) and 647 dossiers (17% of total dossier submissions) contained *in vivo* information solely on a read-across substance derived from Table 2.1. A trend towards greater use of read-across can be observed from table 3 of the main text which shows that for dossiers analysed for this report, 604 read-across ESRs for *in vivo* skin irritation/corrosion were submitted (by the previous registration deadline, 1 113 *in vivo* read-across ESRs were submitted), which shows an increase in the submission of *in vivo* read-across studies. It should be noted that registrants did not always correctly indicate their use of read-across; hence the use of read-across may actually be higher than presented in this report[4].

Registrants are also making use of (Q)SARs and calculations to a limited extent. In the majority of the cases, the registrants have used (Q)SARs as supporting information together with *in vitro* studies, or read-across studies. Only in limited cases have the registrants proposed to fulfil the standard information requirements by solely providing (Q)SAR estimations.

2 During the data extraction, the content of six dossiers was not accessible (due to technical reasons) and therefore their content was not analysed.

3 Table 3 HH endpoint study records provides a number of 1162 total ESRs for *in vitro* of which some were proposals to omit the study. Due to more detailed manual analysis performed for the substance approach, more *in vitro* studies (22) were identified (improper flagging done by the registrants).

4 If the study has been flagged as an "experimental study", but the CAS or EC number reported under test material section (section 1.2) does not correspond to the registered substance, then the study has not been identified as read-across in the data analysis performed for this report.

Table 2.1: Type of dossiers submitted for skin irritation/corrosion endpoint

	Number of dossiers	% of total dossier number	Dossier and ESR ratio[1]
Dossiers with only *in vitro*	316	8.5%	1:1.5
Dossiers with *in vitro* and old *in vivo*[2]	213	5.5%	1:1.5 for *in vitro* 1:3 for *in vivo*
Dossiers with *in vitro* and read-across *in vivo*[3]	111	3.0%	1:2 for *in vitro* 1:3 for *in vivo*
Dossiers with *in vitro* and new *in vivo*	95	2.5%	1:1.5 for *in vitro* 1:1 for *in vivo*
Dossiers with old *in vivo*[4]	2 225	58.5%	1:3
Dossiers with solely *in vivo* read-across data[5]	536	14%	1:3
Dossiers with new *in vivo* for the registered substance	93	2.5%	1:1
Dossiers with only (Q)SARs, or estimations by calculation	18	0.5%	1:1.5
Dossiers with only waiving statements	200	5%	1:1

Note: *in vitro* studies have mainly been performed with the registered substance, but may also contain read-across *in vitro* studies. Differentiations between these have not been made for the purpose of this analysis.

1 These ratios are approximate values for illustrative purposes.
2 Dossiers contain at least one old *in vivo* study performed with the registered substance, but may also contain studies performed with read-across substance, (Q)SARs and waiving statements.
3 In the dossier, all *in vivo* studies have been performed with a read-across substance, but may also contain ESRs for (Q)SARs and waiving statements.
4 Dossiers contain at least one old *in vivo* study performed with the registered substance, but may also contain studies performed with read-across substance, (Q)SARs and waiving statements.
5 In the dossier, all *in vivo* studies have been performed with a read-across substance. May also contain ESRs for (Q)SARs and waiving statements.

Appendix 3: Serious eye damage/Eye irritation

As with the skin irritation/corrosion endpoint (see Appendix 2), studies on serious eye damage/eye irritation are used to predict the local effects of the test substance on human eyes following a single exposure. For *in vivo* studies conducted according to EU TMR/OECD TG standard protocols, the substance to be tested is applied in a single dose to the eye of an experimental animal for 24 hours, usually the albino rabbit. The untreated eye of the test animal serves as the control. The effects of the substance on the exposed animals are usually monitored for 72 hours up to 21 days and reported in a standardised format.

The potential of a substance to cause serious eye damage or eye irritation can be assessed using an *in vitro* test for registration(s) at less than 10 tonnes per year (Annex VII) and with an *in vivo* study at or above 10 tonnes per year (Annex VIII) unless it is possible to conclude on the classification of the substance by using *in vitro* test methods, the substance is a strong acid or base, or it is flammable in air at room temperature. The standard information requirements, and the possibilities to adapt them according to column 2 of Annexes VII and VIII for serious eye damage/eye irritation under REACH, are similar to those for skin irritation/corrosion. In addition, it is possible to adapt the standard information requirements by using general rules for adaption according to Annex XI of the REACH Regulation, given that conditions are met.

There are *in vitro* methods that have undergone a validation process that could be used by registrants to fulfil information requirements for this endpoint. A positive or a negative outcome from *in vitro* assays such as the bovine corneal opacity and permeability (BCOP) or isolated chicken eye (ICE) tests is sufficient to classify substances as inducing serious eye damage (Category 1) or as not requiring classification (No Category) under Annex VII and Annex VIII using adaptations of the standard testing regime specified in Annex XI. A positive outcome from *in vitro* assays such as the fluorescein leakage test method is sufficient to classify substances as inducing serious eye damage (Category 1).

In Table 3 of the main text, which shows the ESRs for serious eye damage/eye irritation, the total number of entries for this endpoint for *in vitro* tests is summarised.

As presented in Figure 3.1, for dossiers for phase-in substances between 100 – 1 000 tonnes per year, 500 (100%) entries have been counted for *in vitro* studies for the endpoint in total. Experimental studies have been used for 348 (69.7%) of all ESRs for this endpoint. A read-across approach is the second most commonly used approach for the *in vitro* serious eye damage/eye irritation endpoint where registrants used this in 93 (18.6%) cases. In 51 (10.2%) of the entries, a weight of evidence

approach was used. Seven of the registrants have proposed to omit the *in vitro* study and (Q)SAR was used in one entry.

When comparing the *in vitro* entries for phase-in substances between 100 – 1 000 tonnes per year with the previous report, the total number of entries submitted has increased substantially (27 *in vitro* ESRs were submitted by the previous registration deadline). Comparison of the distribution of options to fulfil information requirements for *in vitro* serious eye damage/eye irritation among ESRs with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 3) did not result in significant differences.

In contrast with the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of *in vitro* ESRs was around 30% higher (250 vs 172 ESR entries). Comparison of the distribution of options to fulfil information requirements for *in vitro* serious eye damage/eye irritation among the ESRs with the results for dossiers at or above 1 000 tonnes per year described above (see Table 3 of the main text) showed a relative decrease of 8.6% for ESRs with experimental studies and relative increases in read-across (3.4%) and weight of evidence (4.7%) approaches.

For the non-phase in substances produced at or above 100 tonnes per year, 28 *in vitro* serious eye damage/eye irritation ESRs were submitted in total (by the previous registration deadline only one ESR was provided). For these substances, registrants have mainly used the option experimental study (75.0%) and read-across approaches (25.0%) (see Table 3 of the main text).

As presented in Figure 3.2, for dossiers for phase-in substances between 100 – 1 000 tonnes per year, 3 691 (100%) entries have been counted for *in vivo* studies for the endpoint in total. Experimental studies have been used for 1 861 (50.4%) of all ESRs for this endpoint. A read-across approach is the second most commonly used approach for the *in vivo* serious eye damage/eye irritation endpoint where in 982 (26.6%) cases, a read-across approach was followed by the registrants. In 489 (13.2%) of the entries, a weight of evidence approach was used. In 304 (8.2%) of the total entries, the registrants have

Serious eye damage/Eye irritation – *in vitro* (HH)		
	No. ESR	% ESR
ES	348	69.6
TP	0	0.0
RA	93	18.6
FO	7	1.4
WE	51	10.2
QS	1	0.2
MS	0	0.0
Total	500	100

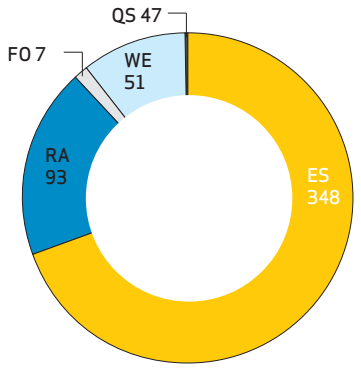


Figure 3.1: Serious eye damage/eye irritation *in vitro* (1 870 dossiers covering phase-in substances 100 - 1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

proposed to omit the *in vivo* study. (Q)SARs were used in 33 (0.9%) and other information has been used in 22 (0.6%) cases.

When comparing the *in vivo* entries for phase-in substances between 100 – 1 000 tonnes per year with the previous report, the total number of submitted *in vivo* entries has increased by 3 167 ESRs. Comparison of the distribution of the options to fulfil information requirements with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 3 of the main text) resulted in a relative decrease of 15.0% in the use of experimental studies and relative increases in read-across (7.1%) and weight of evidence (9.6%) approaches.

In contrast with the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of *in vivo* ESRs was around 20% higher (5 254 vs 4 221 ESR entries). Comparison of the distribution of options to fulfil information requirements for *in vivo* serious eye damage/eye irritation among ESRs with the results for dossiers at or above 1 000 tonnes per year described above (see Table 3 of the main text) did not result in significant differences.

For the non-phase in substances produced at or above 100 tonnes per year, 299 *in vivo* serious eye damage/eye irritation ESRs were submitted in total which shows a 50% increase in the total number of ESRs submitted compared to the previous report (see Table 3 of the main text). Comparison of the distribution of options to fulfil information requirements showed relative increases in the use of experimental studies (15.5%), weight of evidence (2.7%) and read-across (10.3%) approaches. The use of other information and proposals to omit the study showed relative decreases (25.2% and 3.4%, respectively).

Substance approach

A more detailed analysis has been performed for this endpoint due to the developments and international acceptance of alternative test guidelines. For this endpoint, the assessment has concentrated more on the substance level to obtain an understanding of how registrants used alternative test methods to fulfil the standard information requirements for their substances. For this substance level analysis,

Serious eye damage/Eye irritation – *in vivo* (HH))		
	No. ESR	% ESR
ES	1 861	50.4
TP	0	0.0
RA	982	26.6
FO	304	8.2
WE	489	13.2
QS	33	0.9
MS	22	0.6
Total	3 691	100

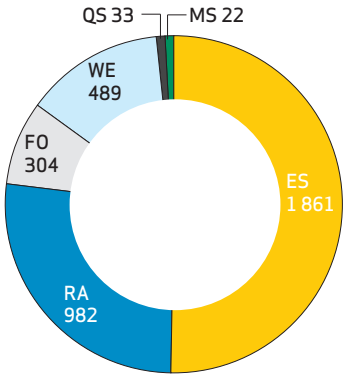


Figure 3.2: Serious eye damage/eye irritation *in vivo* (1 870 dossiers covering phase-in substances 100 – 1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

a cut off year of 2010 has been chosen to identify new *in vivo* studies. The reason for deviating from the cut off year of 2009 specified in section 5.6 of this report, is due to the fact that the BCOP and ICE test methods were only adopted in September 2009 by the OECD. Therefore, for this particular analysis, a cut off year of 2010 was used in order to allow registrants to take these new developments into account and to assess how registrants have made use of these new test methods.

In total, 3 807 dossiers[5] were analysed for this endpoint (in the previous report, 1 862 dossiers were analysed). Table 3 of the main text provides information on the types of strategies that the registrants have chosen to fulfil their standard information requirements for the serious eye damage/eye irritation endpoint. In total, 834[6] *in vitro* endpoint study records (ESRs) were submitted by the 2013 registration deadline (200 *in vitro* ESRs were submitted by the previous registration deadline). The number of ESRs provided by the second registration deadline has increased by more than 100%. This shows that registrants are using alternatives to animal testing much more than they were in the data analysed in 2011.

In 134 dossiers, the information requirement was fulfilled solely by using *in vitro* methods (3.6% of the total dossier submissions). *In vitro* methods were also extensively used together with old *in vivo* studies, as supporting information to read-across approaches (together in 281 dossiers and 7.3% of the total dossier submissions). This shows that registrants have understood the value of *in vitro* studies for providing supporting evidence when only old *in vivo* data is available and when a read-across approach is followed. When new *in vivo* studies have been performed for the registered substance in addition to the *in vitro* studies (5.5% of the total dossier submissions), the registrants seem to follow the correct approach in that when the results from the *in vitro* studies did not allow conclusions to be made on the classification, an *in vivo* study was subsequently performed (currently it is not always possible to cover the whole serious eye damage/eye irritation endpoint solely by using *in vitro* studies).

The registrants have also made use of read-across approaches (category or analogue approaches) and 608 dossiers (16% of total dossier submissions) contained *in vivo* information solely on read-across substances (derived from Table 3.1). A trend towards greater use of read-across can be observed from Table 3 of the main text which shows that for the dossiers analysed for this report, 2 163 read-across ESRs for *in vivo* serious eye damage/eye irritation were submitted (884 *in vivo* read-across ESRs were submitted in the data pool analysed in 2011), which shows an increase of more than 100% in the submission of *in vivo* read-across studies. It is to be noted that registrants have not always correctly flagged their use of read-across; hence the use of read-across may actually be higher than presented in this report[7].

The registrants are also making use of (Q)SARs to a limited extent. In the majority of the cases, the registrants have used (Q)SARs as supporting information together with *in vitro*/ex vivo studies, or read-across studies. Only in a limited number of cases have the registrants proposed to fulfil the standard information requirements by solely providing (Q)SAR estimations. ECHA notes that (Q)SAR estimations could be more helpful when identifying substances requiring classification, but less useful to provide alerts on non-irritant substances.

5 During the data extraction, the content of six dossiers was not accessible (due to technical reasons) and therefore their content was not analysed.

6 Table 3 provides a number of 769 ESRs in total for *in vitro* of which some were proposals to omit the study. Due to more detailed manual analysis performed for the substance approach, more *in vitro* studies (74) were identified (improper flagging done by the registrants).

7 If the study has been flagged as an "experimental study", but the CAS or EC number reported under test material section (section 1.2) does not correspond to the registered substance, then the study has not been identified as read-across in the data analysis performed for this report.

Table 3.1: Type of dossiers submitted for eye irritation endpoint

	Number of dossiers	% of total dossier number	Dossier and ESR ratio[1]
Dossiers with only *in vitro*	135	3.5%	1:1
Dossiers with *in vitro* and old *in vivo*[2]	149	3.9%	1:1.5 for *in vitro* 1:3 for *in vivo*
Dossiers with *in vitro* and read-across *in vivo*[3]	130	3.4%	1:1.5 for *in vitro* 1:2 for *in vivo*
Dossiers with *in vitro* and new *in vivo*[4]	210	5.5%	1:1 for *in vitro* 1:1 for *in vivo*
Dossiers with old *in vivo*[5]	2108	54.4%	1:3
Dossiers with solely *in vivo* read-across data[6]	608	16.0%	1:3
Dossiers with new *in vivo* for the registered substance	153	4.0%	1:1
Dossiers with only (Q)SARs, or estimations by calculation	18	0.5%	1:1
Dossiers with only waiving statements	295	8.1%	1:1

Note: *in vitro* studies have mainly been performed with the registered substance, but may also contain read-across *in vitro* studies. Differentiations between these have not been made for the purpose of this analysis.

1 These ratios are approximate values for illustrative purposes
2 Dossiers contain at least one old *in vivo* study performed with the registered substance, but may also contain studies performed with a read-across substance, (Q)SARs and waiving statements.
3 In the dossier, all *in vivo* studies have been performed with a read-across substance, but may also contain ESRs for (Q)SARs and waiving statements.
4 Dossiers may also contain old *in vivo* studies performed with the registered substance, or with a read-across substance and (Q)SARs
5 Dossiers contain at least one old *in vivo* study performed with the registered substance, but may also contain studies performed with a read-across substance, (Q)SARs and waiving statements.
6 In the dossier, all *in vivo* studies have been performed with a read-across substance. May also contain ESRs for (Q)SARs and waiving statements.

Appendix 4: Skin sensitisation

Skin sensitisation is the toxicological endpoint associated with chemical substances that have the intrinsic property to cause skin sensitisation resulting in allergic contact dermatitis in humans following repeated exposures to a substance.

The standard skin sensitisation test methods, for which EU TMR/OECD TG are available, include the murine local lymph node assay (LLNA), the guinea pig maximisation test (GPMT) and the occluded patch test of Buehler. In the LLNA, the test substance is applied to the ears of mice for three days and later tritiated thymidine is injected intravenously to measure cell proliferation in auricular lymph nodes. An increase in lymph node cell proliferation compared to control animals indicates sensitisation.

In the GPMT, guinea pigs are exposed to the test substance by intradermal injection and topical application by occlusion. Following a rest period of 10 to 14 days, the challenge dose is applied topically under 24 hours occlusion. The extent and degree of skin reactions to this challenge exposure are then compared with control animals.

In the Buehler test, guinea pigs are repeatedly exposed to the test substance by topical application under occlusion. Following a rest period of 12 days, a dermal challenge treatment is performed under occlusive conditions. Skin reactions to the challenge exposure are compared with those in control animals.

There are currently validation activities ongoing for non-animal alternative methods to identify skin sensitisation hazard potential. The aim of these validation activities is to assess the performance of such methods in terms of reproducibility and predictive capacity as potential components of non-animal integrated approaches for skin sensitisation testing.

The information requirements for skin sensitisation are described in REACH Annex VII. Data on skin sensitisation are required for substances produced or imported at or above one tonne per year, and hence should be in all the registrations considered for the purpose of this report. *In vivo* studies do not need to be conducted, if there is enough evidence that the substance should be classified or on the basis of its physicochemical properties (strong acid or base or flammable in air at room temperature). The murine local lymph node assay (LLNA) is the first choice method for *in vivo* testing and another test should only be chosen in exceptional circumstances that have to be scientifically justified. The LLNA is regarded as being more capable of predicting the relative potency of skin sensitising chemicals (the chemical's relative power/strength to induce skin sensitisation).

In Table 3 of the main text, which shows the ESRs for skin sensitisation, the total number of entries for this endpoint is summarised.

As presented in Figure 4.1, for dossiers for phase-in substances between 100 – 1 000 tonnes per year, 3 565 (100%) entries have been counted for *in vivo* studies for the endpoint in total. Experimental studies have been used for 1 525 (42.8%) of all ESRs for this endpoint. A read-across approach is the second most commonly used approach for the *in vivo* skin sensitisation endpoint where registrants used it in 1 029 (28.9%) cases. In 678 (19.0%) of the entries, a weight of evidence approach was used. In 254 (7.1%) of the total entries, the registrants have proposed to omit the *in vivo* study. (Q)SARs were used in 52 (1.5%) and other information has been used in 27 (0.8%) cases.

When comparing the *in vivo* entries for phase-in substances between 100 – 1 000 tonnes per year with the previous report, the total number of submitted *in vivo* entries has increased by 3 077 ESRs. Comparison of the distribution of the options to fulfil information requirements with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 3 of the main text) resulted in a relative decrease of 15.8% in the use of experimental studies. The use of other information also decreased by 1.5%. Relative increases were noted in read-across (4.5%), weight of evidence (4.3%) approaches and proposals to omit the *in vivo* study (7.1%).

In contrast to the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of *in vivo* ESRs was around 20% higher (4 657 vs 3 754 ESR entries). Comparison of the distribution of options to fulfil information requirements for *in vivo* skin sensitisation among ESRs with the results for dossiers at or above 1 000 tonnes per year described above (see Table 3 of the main text) did not result in significant differences.

For non-phase-in substances produced at or above 100 tonnes per year, 299 *in vivo* skin sensitisation ESRs were submitted in total which shows around a 40% increase in the total number of ESRs submitted (see Table 3). Comparison of the distribution of options to fulfil information requirements showed

Skin sensitisation – *in vivo* (HH)		
	No. ESR	% ESR
ES	1 525	42.7
TP	0	0.0
RA	1 029	28.9
FO	254	7.1
WE	678	19.0
QS	52	1.5
MS	27	0.8
Total	3 565	100

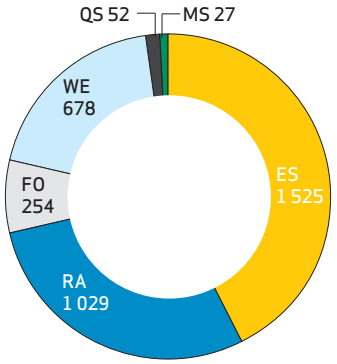


Figure 4.1: Skin sensitisation *in vivo* (1 870 dossiers covering phase-in substances 100 – 1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

a relative increase in the experimental study (24.7%), weight of evidence (7.4%) and read-across (1.4%) approaches. The use of other information and proposals to omit the study showed relative decreases (19.4% and 14.9%, respectively).

In addition to the ESR approach described above, the dossiers where *in vitro/in chemico* methods were provided were analysed using a substance level approach due to the ongoing validation of alternative approaches for this endpoint. In total, 65[1] *in vitro* ERS entries covering 34 dossiers where submitted. For eight of the 34 dossiers, the skin sensitisation endpoint was covered solely with *in vitro/in chemico* studies. In 10 of the 34 dossiers *in vitro/in chemico* studies were provided to support a read-across approach. In 21 out of the 34 dossiers, *in vivo* studies were provided in addition to the *in vitro/in chemico* studies. In addition, (Q)SAR estimations were provided to support their selected approach in four of these cases.

In the majority of the cases when *in vitro/in chemico* studies have been submitted, the registrants have used a weight of evidence approach. The results show that registrants have used alternative approaches more frequently in comparison to the last report to fulfil the information requirements for this endpoint. The quality of the submitted information has not been evaluated for the purpose of this report.

1 Table 3 provides a total number of 75 ESRs for *in vitro* methods. Due to more detailed manual analysis of these entries, it was revealed that 10 of these ESRs were incorrectly flagged as *in vitro*, hence the differences in the ESR numbers.

Appendix 5: Repeated dose toxicity

Information on repeated dose toxicity is used to predict the effects of longer term exposure of chemical substances to humans. During the study, purpose-bred animals such as the rat or mouse receive repeated doses of a substance through oral, dermal or inhalation routes of exposure. In Annex VIII (10-100 tpa), a study with a duration of 28 days (sub-acute) is the standard information requirement, but note as described below that there was the possibility to omit this study from the core dataset at the time of registration to achieve a technically complete dossier by making a testing proposal for a 90-day study if adequate risk management measures are in place. In Annex IX (100-1 000 tpa), additionally a study with a 90-day duration (sub-chronic) is the standard information requirement. The oral route in many cases is the default, but depending on the relevant exposure route for humans, dermal application or inhalation may also be needed. In Annex X (>1 000 tpa), long-term (chronic) studies can be proposed by the registrant or can be used to fill the endpoint.

In the IUCLID dossier, this endpoint can have entries for studies with different durations and for different routes and for all combinations of these. In addition, a so-called combined screening study, combining studies of repeated dose toxicity with reproductive toxicity, may have been provided by the registrant to meet the core data requirements (Annex VIII) for this endpoint. As already stressed in the first report, *in vitro* methods have not been validated for repeated dose toxicity and cannot be predicted by (Q)SAR. Alternative methods are therefore mainly other prediction methods (read-across and grouping), weight of evidence approaches and the possibility to omit the studies in accordance with the requirements in column 2 of Annexes VII to X and in Annex XI.

The results of data analysed in 2011 demonstrated a clear difference between the Annex X and Annex IX dossiers, as for Annex X registrations this endpoint had almost 10 times more ESRs than for Annex IX dossiers (due to different standard information requirements), while when analysing a current dossier pool, this proportion has changed. In Table 4, summarising ESRs for repeated dose toxicity, the total number of entries for this endpoint separated by route and duration is collected.

In Figure 5.1 for the dossiers for phase-in substances between 100 – 1 000 tonnes per year, 9 786 entries (100%) have been counted in total as ESRs. In 2 411 cases (24.6%), registrants used experimental data to cover these endpoints. In 227 (2.3%) ESRs, testing proposals have been submitted for these phase-in substances between 100 – 1 000 tonnes per year. Read-across approaches have been used in 3 320 (32.9%) of the ESRs. A weight of

evidence approach was flagged by the registrants in 1 051 entries (14.0%). Proposals to omit the information have been set by the registrants in 2 435 cases (24.9%). (Q)SAR predictions are not very relevant to these endpoints and have thus only been used in 36 cases (0.4%). Other information has been used in 85 cases (0.9%). It should be noted that this analysis covers all routes and all study durations.

With regard to the updated pool of dossiers for phase-in substances at or above 1 000 tonnes per year, the registrants used different approaches (including alternatives to animal testing) to fulfil the information requirements for this endpoint in a rather similar way as reported in the previous report in 2011. Due to updated dossiers and late registrations, the number of ESRs available for this endpoint by the cut off date of analysis (1 October 2013) increased by 2 248 (13 038 by 1 October 2013 and 10 790 in 2011, respectively), while the relative distribution among the approaches (expressed as a percentage) remained similar. More noticeable differences on choosing read-across, weight of evidence approaches (relative percentage increase at 10.6% and 7.0% in 2013, respectively) and omitting or providing information as other studies (relative percentage decrease at 8.2% and 13.4% by 1 October 2013, respectively), were observed for non-phase-in substances. Detailed information on the overall distribution of data, including results from 2011, is provided in Table 4 of the main text.

RDT – all routes, all study durations (HH)		
	No. ESR	% ESR
ES	2 411	24.6
TP	227	2.3
RA	3 220	32.9
FO	2 435	24.9
WE	1 372	14.0
QS	36	0.4
MS	85	0.9
Total	9 786	100

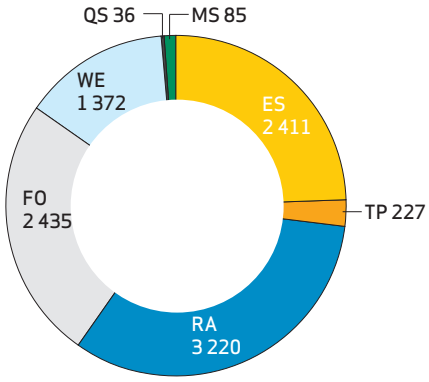


Figure 5.1: Repeated dose toxicity – all routes, all study durations (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 6: Genetic toxicity

The aims of testing for genetic toxicity (genotoxicity) are to assess the mutagenic potential 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 ability of substances to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity). To obtain such information, a number of *in vitro* and *in vivo* test methods officially adopted by the EU or the OECD are available. Non-testing options, for example, (Q)SAR and the use of read-across approaches, may also provide information on the mutagenic potential of chemical substances.

Standard information requirements on mutagenicity under REACH are described in Annexes VI to X and the specific rules to omit, replace, and adapt the required standard data or to use alternative options are listed in column 2 of Annexes VII to X.

For substances manufactured or imported at the lower tonnage (1 - 10 tonnes per year), only an *in vitro* gene mutation study in bacteria is required (Annex VII). No further studies at this tonnage level are required if the result is negative (i.e. no signs of adverse effects).

For substances falling under the Annex VIII information requirements of REACH (10 - 100 tonnes per year), additional *in vitro* tests are required. An *in vitro* cytogenicity study or an *in vitro* micronucleus study in mammalian cells needs to be conducted but may be omitted if reliable data from an *in vivo* cytogenicity test are available or if the substance is already classified as a carcinogen Cat 1A or 1B or germ cell mutagen Cat 1A, 1B or 2. If both the *in vitro* gene mutation study in bacteria and the cytogenicity study in mammalian cells are negative, another *in vitro* study – gene mutation in mammalian cells – is required, unless reliable *in vivo* mammalian gene mutation data are available. At this tonnage level, *in vivo* mutagenicity studies shall only be considered in cases of a positive (i.e. signs of an adverse effect) result in any of the required *in vitro* tests.

For substances manufactured or imported between 100 - 1 000 tonnes per year, if there is a positive result in any of the *in vitro* genotoxicity studies and no reliable *in vivo* data available, registrants have to submit a testing proposal for an *in vivo* somatic cell genotoxicity study. For substances falling under the Annex X requirements of REACH, a positive result in any *in vitro* studies may additionally trigger a need for a second *in vivo* somatic cell test. For all substances manufactured at 100 tonnes per year or more, a positive outcome from *in vivo* somatic cells test should lead to considerations on the potential for germ cell mutagenicity. The new OECD TG 488 ('Transgenic Rodent Somatic and Germ Cell

Gene Mutation Assays') was adopted in July 2011 and updated in July 2013. The 'Comet assay' draft TG has been submitted to the 26th Meeting of the OECD Working Group of National Coordinators of the Test Guidelines Programme (WNT) for approval in April 2014.

In summary, generally only *in vitro* mutagenicity tests are needed for the core data, and, depending on available *in vitro* data, some *in vivo* follow-up mutagenicity studies may be necessary as higher-tier studies to be conducted after the testing proposals have been approved.

In Table 4 on the ESRs for genetic toxicity, the total number of entries for this endpoint separated by *in vitro* and *in vivo* test is summarised.

As presented in Figure 6.1, for the dossiers for phase-in substances between 100 - 1 000 tonnes per year, 10 083 entries (100%) have been counted in total as ESRs. Experimental studies have been used for 4 267 (42.3%) of all ESRs for this endpoint. Read-across appeared to be the main alternative method selected to avoid unnecessary testing for this endpoint: in 3 073 ESRs (30.5%), a read-across approach was followed by the registrants. The weight of evidence approach was chosen in 2 210 (21.9%) of all ESRs and proposals to omit a study have been found in 371 (3.7%) of the ESRs. (Q)SAR approaches were used in 92 ESRs, and other information has been used in 70 (0.7%) cases.

In contrast with the previous report, for the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of ESRs was only around 20% higher (12 808 vs 10 322 ESR entries) than at the lower tonnage described above. Relative comparison of the distribution of options to fulfil information requirements among ESRs with the results for dossiers at 100 – 1 000 tonnes per year described above (see Table 4 of the main text) did not result in the observation of significant differences, with the exception of a relative increase in the use of experimental data (57.4% of ESRs for substances produced at or above 1 000 tonnes per year) and a relative decrease in the use of weight of evidence approaches (12.9% ESRs for highest tonnage grade).

For the non-phase-in substances produced at or above 100 tonnes per year, the results were slightly

Genetic toxicity - *in vitro* (HH)		
	No. ESR	% ESR
ES	4 267	42.3
TP	0	0.0
RA	3 073	30.5
FO	371	3.7
WE	2 210	21.9
QS	92	0.9
MS	70	0.7
Total	10 083	100

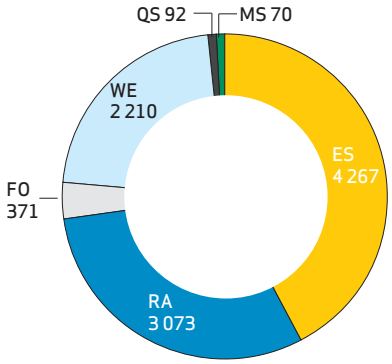


Figure 6.1: Genetic toxicity *in vitro* (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

different. The total number of ESRs submitted by 1 October 2013 was almost 50% less than in the data pool analysed in 2011, while the percentage of experimental studies slightly increased in 2013 (14.3%). The read-across approaches have now been proposed in 22.7% of the ESRs, while in the previous data pool, this approach was only chosen in 10.3% of the cases. For non-phase-in substances registered in 2013, the registrants have used relatively fewer other options to fulfil information requirements than before (1.2% and 26% of the ESRs, respectively).

As presented in Figure 6.2, the registrants submitted a total of 2 254 ESRs (100%) of genetic toxicity *in vivo* studies to fulfil the information requirements for the phase-in substances manufactured or imported between 100 and 1 000 tonnes per year. The distribution among the different options to fulfil the information requirements under REACH seemed to be similar to the genetic toxicity *in vitro* endpoint (notably, for the same tonnage band). Experimental studies have been used for 837 (37.1%) of all ESRs for this endpoint. Read-across approaches have been proposed in 693 (30.7%) cases. In 506 (22.4%) of these entries, a weight of evidence approach was chosen by the registrants.

For the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of ESRs was slightly higher (4 281 vs 3 532 ESR entries) when compared with the pool of dossiers for substances of this tonnage band analysed in 2011, but no significant differences in the proportional distribution of various options of ESRs were observed.

Regarding the dossiers for the non-phase-in substances at or above 100 tonnes per year, more than 50% of the ESRs have been filled with data from experimental studies, and the read-across approach was flagged in 23.3% of the cases, resulting in a relative percentage increase of the use of this option by 18.0% (when compared to data analysed in 2011). In 2013, only three ESRs were filled by other information (1.7%), while in 2011 this option was found in 36.2% of the ESRs.

Genetic toxicity – *in vivo* (HH)		
	No. ESR	% ESR
ES	837	37.1
TP	39	1.7
RA	693	30.7
FO	128	5.7
WE	506	22.4
QS	25	1.1
MS	26	1.2
Total	2 254	100

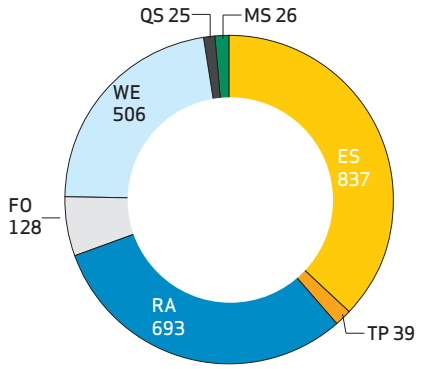


Figure 6.2: Genetic toxicity *in vivo* (1 882 dossiers covering phase-in substances 100-1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 7: Toxicity to reproduction

The aims of testing and standard information requirements under REACH regarding toxicity to reproduction were already summarised and addressed in the first report, published by ECHA in 2011. In brief, this information is based on separate testing performed for two related endpoints: a prenatal developmental toxicity study analysing possible damaging effects on the developing organism and a reproduction 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 and therefore evaluate potentially very serious consequences for human reproduction as well as foetal and child development. The standard laboratory animals used for these study types are rat, rabbit or mouse for the developmental tests or rat and mouse for the reproduction studies. Alternative *in vitro* tests or computational prediction methods are currently unable to predict the impact that disturbing single or multiples of these mechanisms could have on the entire reproductive process including the normal postnatal development.

Standard information requirements on toxicity to reproduction under REACH apply for the substances manufactured on imported at or above 10 tonnes per year (Annex VIII to X substances) and get more exhaustive for higher production tonnages.

For the substances of 10 - 100 tonnes per year, a reproduction/developmental toxicity screening test (ref. OECD TG 421 or 422) is usually required to meet the core data requirements. These screening tests cannot be used as an alternative or replacement for the higher-tier studies on reproductive toxicity. However, should the screening study demonstrate clear adverse effects on reproduction functions or reproductive organs and provided that the results are sufficient for classification and risk assessment, there may be no need for further testing.

For substances manufactured or imported between 100 - 1 000 tonnes per year, in addition, a prenatal developmental toxicity study in one species, rat or rabbit (ref. OECD 414, EU B.31) is required. As already noted in the first report, since the information for developmental toxicity in one species is required by both Annexes IX and X and the requirements are additive, the requirements from these two annexes comprise pre-natal developmental toxicity tests in two species. However, the legislation outlines that the decision on the need to perform the test in a second species should be based on the outcome of the study on the first species and all other relevant data.

For substances falling under Annex X requirements of REACH, in addition to the previously outlined tests, a two-generation reproduction toxicity

study (ref. OECD TG 416, EU B.35) is required. If the 28-day repeated dose toxicity or the 90-day repeated dose toxicity studies indicates adverse effects on reproductive organs or tissues, a two-generation reproduction toxicity study can already be performed at the Annex IX level.

In Table 4 of the main text, which shows the endpoint study records (ESRs) for reproductive toxicity, the total number of entries for this endpoint (toxicity to reproduction and developmental toxicity) is summarised.

As presented in Figure 7.1 for dossiers for phase-in substances between 100 and 1 000 tonnes per year, registrants submitted 3 868 entries as ESRs – many more than reported in 2011 (487 ESRs only). Experimental data have been used for 768 (19.9%) of the ESRs. The proposals to omit information have been used in 1 365 (35.3%) entries, and a read-across approach was selected in 1 005 ESRs (26.0%). Registrants also submitted 62 testing proposals to fulfil information requirements for this endpoint. (Q)SAR predictions were used in 40 ESRs, and miscellaneous studies were submitted in 23 cases.

For the dossiers of phase-in substances produced at or above 1 000 tonnes per year, the total number of ESRs was 973 higher than in the data analysed in 2011. No significant percentage differences among selected options to fulfil information requirements with the results for dossiers at or above 1 000 tonnes per year analysed in 2011 were noted.

For the non-phase-in substances produced at or above 100 tonnes per year, registrants submitted two times more ESRs than found in the data analysed in 2011 for such substances (156 in 2011 and 327 in the current data pool, respectively). The relative percentage distribution of different approaches for filling IUCLID dossiers did not vary too much from those reported in the 2011 report, with the exception of an increased use of read-across (17.7% of ESRs) and weight of evidence (12.2% of ESRs) approaches.

As reported in Figure 7.2, regarding prenatal developmental toxicity, registrants have submitted 4 217 entries for phase-in substances between 100 and 1 000 tonnes per year – more than seven times more ESRs for this tonnage band in comparison with

Toxicity to reproduction (HH)		
	No. ESR	% ESR
ES	768	19.9
TP	62	1.6
RA	1 005	26.0
FO	1 365	35.3
WE	605	15.6
QS	40	1.0
MS	23	0.6
Total	3 868	100

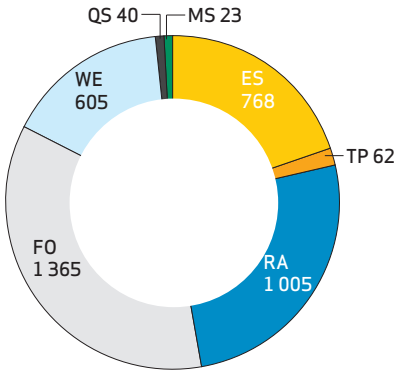


Figure 7.1: Toxicity to reproduction (1 882 dossiers covering phase-in substances 100-1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

the data analysed for the 2011 report. 858 ESRs were filled by experimental data (20.4% of the entries). In line with the findings reported in 2011, this endpoint was again the leading endpoint for testing proposals – this time registrants submitted 293 testing proposals for Annex IX dossiers, corresponding to 6.9% of the ESRs. In 1 582 (37.5%) entries, registrants flagged a read-across approach. Proposals to omit the study and to use a weight of evidence approach have been selected in 15.8% and 17.7% of ESRs, respectively. 40 entries were filled by (Q)SAR predictions. In comparison to the approaches chosen by registrants as described in the first report, a relative decrease in the percentage of experimental studies was observed, while use of read-across, weight of evidence and possibilities to omit the study increased. Detailed numerical and percentage distribution is provided in Table 4 of the main text.

For the dossiers of phase-in substances produced at or above 1 000 tonnes per year, 5 149 ESR entries were extracted from the IUCLID database. In 2 047 (39.8%) of the cases, registrants referred to the experimental studies and submitted 166 testing proposals. A read-across approach has been chosen in 31.4% of the ESRs. In general, the approaches to fulfil information requirements for this tonnage band were closely similar to the ones reported in 2011.

The percentage of experimental studies for non-phase-in substances at or above 100 tonnes per year reached 32.7% and the read-across approaches have been chosen in 24.3% of the ESRs. IUCLID flags to omit the study have been chosen in 24.7% of the cases. The most significant relative percentage differences were noted with regard to the use of read-across, weight of evidence approaches and possibilities to omit studies.

Development toxicity (HH)		
	No. ESR	% ESR
ES	858	20.4
TP	293	6.9
RA	1 582	37.5
FO	668	15.8
WE	745	17.7
QS	40	0.9
MS	30	0.7
Total	4 216	100

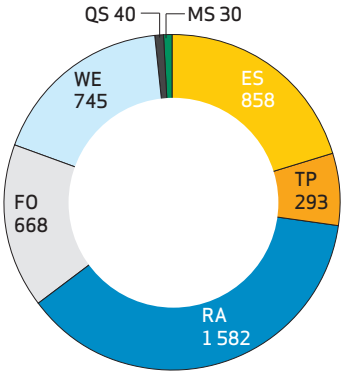


Figure 7.2: Developmental toxicity (1 882 dossiers covering phase-in substances 100-1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 8: Carcinogenicity

As already outlined in the previous report, the objective of carcinogenicity studies on chemical substances is to identify potential human carcinogens, their mode(s) of action and their potency. In general, data from studies of cancer in humans are only available for a limted number of substances and studies using animals are used to assess carcinogenic properties of substances.

Based on the complexity and length of the process of carcinogenesis, complex biological interactions and many different modes of action involved, even for the same substance, it is not yet possible to get a full understanding and complete mimicking through the use of alternative, non-animal tests. The two-year cancer assay in rodents, usually the rat or mouse, is typically conducted to evaluate the cancer hazard and potency of a substance.

REACH requires information to be provided for the carcinogenicity endpoint for the highest tonnage substances (at 1 000 tonnes per year or above, Annex X). Annex X (1 000 tpa) of REACH requires information, or if certain conditions are met the submission of a testing proposal, for the highest tonnage substances (at 1 000 or more tonnes per yeaar). Annex IX (100 to 1 000 tonnes per year) of the REACH Regulation does not specify the need for information on carcinogenicity endpoint. However, dossiers at 100 to 1 000 tpa may contain information on carcinogenicity which the registrants have available or it may be provided by registrants for substances registered at > 1 tpa if they are classify as carcinogenic, mutagenic or reprotoxi. In 2011, most ESR for this endpoint were identified in dossiers covering regitrations at 1 000 or more tpa as these made up the vast majority of the analysed dossiers. In 2013, the greater number of ESR is mainly due to those found in dossiers at 100 to 1 000 tpa and which make up a greater proportion of the datapool that has now been analysed,

As presented in Figure 8.1, for the Annex IX phase-in substances, 1 299 ESRs have been submitted on carcinogenicity. 32.9% of them were experimental studies. In 33.7%, registrants chose a read-across approach, in 14.2% of ESRs registrants chose to omit the study and in 16.2% of the entries, a weight of evidence approach was selected by the registrant. No testing proposals on carcinogenicity for this tonnage band have been submitted. (Q)SAR predictions have been provided eight times.

For the dossiers of phase-in substances produced at or above 1 000 tonnes per year, a total of 4 088 ESR entries were extracted from the IUCLID database in 2013 (3 559 in 2011, respectively). Despite a notable increase in the number of ESRs, the relative percentage distribution for this tonnage level of the approaches used by registrants appeared to

be almost identical to that found in 2011. Detailed numbers and percentage distributions are provided in Table 4 of the main text.

In the current data pool, 94 ESRs on carcinogenicity for non-phase-in substances at or above 100 tonnes per year have been found.

Carcinogenicity (HH)		
	No. ESR	% ESR
ES	427	32.9
TP	0.0	0.0
RA	438	33.7
FO	184	14.2
WE	211	16.2
QS	8	0.6
MS	31	2.4
Total	1 299	100

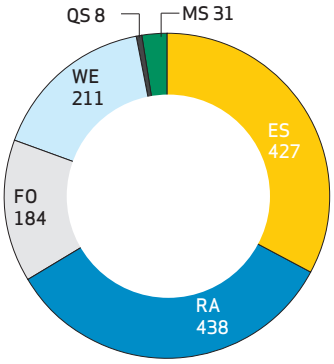


Figure 8.1: Carcinogenicity (1 882 dossiers covering phase-in substances 100-1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 9: Bioaccumulation in fish

Information on accumulation in aquatic organisms is a vital part to understanding the environmental fate and behaviour of a substance. This information is used for hazard classification and PBT assessment as well as wildlife and human food chain exposure modelling for the chemical safety assessment. It is also a factor in deciding whether long-term ecotoxicity testing might be necessary. This is because the accumulation of a chemical substance following long-term exposure, even when external concentrations are very low, may result in internal concentrations of a substance which causes toxicity to the organism. Highly bioaccumulative chemical substances may also be transferred through the food web, which in some cases may lead to biomagnification.

Under REACH, standard information requirements on bioaccumulation in aquatic organisms, preferably fish, are included in Annex IX, and are thus applicable to substances manufactured or imported at or above 100 tonnes per year. Reliable measured data are preferred if available, but the study does not need to be conducted if the substance has low potential for bioaccumulation or direct and indirect exposure of the aquatic compartment is unlikely (REACH Annex IX, Column 2). REACH Annex XI also applies, encouraging the use of alternative information at all supply levels before a new test on fish is conducted.

Prediction techniques are well developed for many classes of organic substances, and surrogate information (for example, the n-octanol-water partition coefficient or Kow) as well as invertebrate tests may sometimes suffice on their own or as part of a weight of evidence approach. In addition, research is currently ongoing to develop an *in vitro* bioaccumulation (metabolism) study. For this analysis, as it was focused only on the use of vertebrates, only those records in which registrants declared the use of fish as the test species were counted, in that ESRs were not counted where either the test species was declared as a species other than fish or was not specified. Therefore, ESRs referring only to invertebrates were not analysed for the purposes of this report. The number of testing proposals was confirmed manually.

Presented below are the data on the ESRs for this endpoint. In addition, trends are considered, where relevant, by reference to Table 5, i.e. the difference in the relative proportion of ESRs (by IUCLID purpose flag), found in 2011 versus 2013.

As presented in Figure 9.1, for the phase-in substances between 100 and 1 000 tonnes per year, registrants have submitted a total of 1 741 ESRs related to the fish bioaccumulation study in the IUCLID database. Of these ESRs, 226 (13%) were filled by experimental data. This represents

a reduction of 8% compared to the previous submission. The reduction is even more substantial when looking at the figures for experimental data for phase-in substances at or above 1 000 tonnes per year (20.1% less than the last submission) and 33.7% less for non-phase-in substances.

A similar trend has been identified for read-across, where the number of entries for phase-in substances between 100 and 1 000 tonnes per year was 298 (20% less than in 2010), for phase-in substances at or above 1 000 tonnes per year was 247 (11.4% less than in the previous data pool) and for non-phase-in substances was 13 (4.5% less than in the previous data pool).

Nevertheless, if the proportion of total ESRs which are experimental studies and read-across is less, the opposite has occurred for the use of a weight of evidence approach and (Q)SARs. In fact, for substances between 100 and 1 000 tonnes per year, a weight of evidence approach was chosen in 992 cases (57% of ESRs) and (Q)SARs in 193 cases (11.1% of the ESRs). These represent an increase of 18.5% and 11.1%, respectively, compared to the previous submission deadline.

The trend is confirmed also when looking at non-phase-in substances, with 27 entries for a weight of evidence approach (21.8% of ESRs, meaning a 16.8% increase compared to 2011) and 39 entries for (Q)SARs (31.5% of ESRs, meaning a 31.5% increase compared to 2011, when the total was "zero"). The proneness towards the use of (Q)SARs is even more evident for substances at or above 1 000 tonnes per year, where 723 ESRs represent 39% of the total and an increase of 35.9% compared to the previous submission. This is also reflected in a 3% decrease in flags for weight of evidence compared to 2011.

Notwithstanding the above, a couple of the cited figures merit a separate consideration. For example, a detailed analysis of the aforementioned findings on the use of (Q)SARs for phase-in substances above 1 000 tonne per year, for which there were 723 entries, revealed how this number is strongly dependant on the nature of the substances that have been registered. In fact, for 20 UVCB substances registrants have used a (Q)SAR to predict the effects of each of the 33 components of their substances for

Bioaccumulation - fish (ENV)		
	No. ESR	% ESR
ES	226	13
TP	9	0.5
RA	298	17.1
FO	0	0
WE	992	57
QS	193	11.1
MS	23	1.3
Total	1 741	100

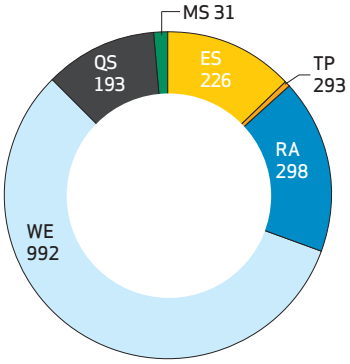


Figure 9.1: Bioaccumulation in fish (1 882 dossiers covering phase-in substances 100-1 000 tpa, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

this endpoint, which means a total of 660 entries out of 723. It follows that a reasonably small group of substances actually contributed to the total figure to such an extent that the initial impression of a large number of registrants having made use of (Q)SARs needs to be reconsidered under the light of this analysis. This phenomenon equally applies for the (Q) SAR values of non-phase-in substances, where the 39 entries that would represent a 31.5% relative increase compared to the number published in 2011 is biased by the presence of one UVCB substance with 33 components for which the registrant applied the approach described above.

A more in-depth analysis of the figures on the use of a weight of evidence approach for Annex IX and X substances, also revealed important information. In fact, following the selection of weight of evidence under the 'purpose flag' field, registrants have then the option to select "(Q)SARs" or "estimated by calculation" in the field 'study result type'. This means that information on the use of (Q)SARs is hidden within the figure for weight of evidence. In particular, out of a total of 1 437 ESRs for weight of evidence, 510 (49 Annex X and 461 Annex IX), i.e. 35.0% of the entries, are single or multiple (Q)SAR study records. Thus, to obtain the true total number of (Q)SAR entries for bioaccumulation we need to add the latter figure to the total number of ESRs for which (Q)SARs were selected as a key study (955 ESRs), thus providing us with a total of 1 465 entries (representing 39.3% of all ESRs for this endpoint). Detailed results including relative differences between both data pools are provided in Table 5 of the main text.

Appendix 10: Toxicity to fish

Information on aquatic toxicity is used to assess the hazards and risks of a test substance to freshwater and marine organisms living in the water column. In addition, the data obtained from testing on aquatic species may also serve as a basis for extrapolation of the effects to other compartments such as sediment and soil. Data on fish toxicity are generated for environmental hazard assessment of substances (i.e. classification and derivation of PNEC) and the estimation of toxicity in the PBT assessment.

Short-term toxicity testing on fish is required for substances covered by Annex VIII of REACH (produced or imported in a quantity of at least 10 tonnes per year). However, this test does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur (for example, the substance is highly insoluble in water or the substance is unlikely to cross biological membranes). However, if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms, long-term testing as described in Annex IX must be considered. Long-term testing should also be considered if the substance is poorly water soluble. Hence, acute fish toxicity is generally part of the core data for all the registered substances in this study whereas long-term fish toxicity is a higher-tier study to be covered in a testing proposal.

Regarding alternative methods covering this endpoint, the Fish Embryo Toxicity (FET) Test has been officially approved as an OECD Test Guideline (OECD 236) in July 2013 (no corresponding EU Test Method is currently available). The applicability of the test for REACH registration purposes is under consideration. Regarding the use of (Q)SARs, please refer to section 1.4 of the report. In addition, there is the possibility to assess toxicity to fish using read-across approaches if scientifically justified.

Presented below are the data on the ESRs for this endpoint. In addition, trends are considered, where relevant, by reference to Table 5, i.e. the difference in the relative proportion of ESRs (by IUCLID purpose flag), found in 2011 versus 2013.

As presented in Figure 10.1, for the short-term toxicity to fish, registrants have submitted 6 104 ESR entries for phase-in substances between 100 and 1 000 tonnes per year (Annex IX). Experimental data were indicated in 2 368 ESRs (38.8% of the entries), which represents a 9.9% reduction compared to the previous submission. On the other hand, the use of experimental data for non-phase-in substances has increased by 5.7%, resulting in 213 entries from a total of 362 ESRs. The number for substances at or above 1 000 tonnes per year has increased by 1.6% when analysing a current data pool accounting for 4 552 entries from a total of 8 917 ESRs.

For Annex IX dossiers, registrants filled 2 154 entries (35.3%) using a read-across approach and 1 094 entries (17.9%) as weight of evidence, indicating a respective increase of 8% for read-across and 1.8% for weight of evidence approaches compared to the figures for the previous report published in 2011. A similar trend has been identified for non-phase-in substances, with a total of 82 entries (22.7%) as a read-across approach and 38 entries (10.5%) as weight of evidence, indicating an increase of 14.3% and 6.3%, respectively, compared to the previous submission. The latter increases are accompanied by a reduction in the number of entries chosen as miscellaneous, with a total of 10 (25.2% less than presented in the previous report).

Although marginal, substances at or above 1 000 tonnes per year displayed a decreasing trend for read-across and choices for omitting the study, respectively 1.2% (1 692 entries submitted by 1 October 2013) and 0.6% (103 entries submitted by 1 October 2013) less than reported in 2011. The use of weight of evidence entries increased by 0.8%.

A positive trend was identified for all three classes of substances with regards to the use of (Q)SARs: dossiers for substances at or above 1 000 tonnes per year contained a total of 330 entries (1.6% more than the previous data pool); dossiers for substances between 100 and 1 000 tonnes per year contained a total of 120 entries (0.7% more than in the previous data pool); dossiers for non-phase-in substances contained a total of 12 entries (1.2% more than in the previous data pool).

As presented in Figure 10.2, registrants have submitted 3 563 ESR entries for long-term toxicity to fish for phase-in substances between 100 and 1 000 tonnes per year. A total of 420 ESRs were filled by experimental data (11.8% of the entries) and 25 ESRs – by testing proposals (0.7% of all entries). These figures account for a 23.7% and 0.5% reduction, respectively, compared to the previous submission deadline.

The most used options were proposals to omit the study and the use of read-across approaches, which have been selected in 42.5% and 28.8% of the ESRs, respectively. The difference with the data assessed in 2011 is a 25.4% increase for the former and interestingly a 6.0% decrease for the latter. Regarding the other options, in 462 (13.0%) of the

Short-term toxicity to fish (ENV)		
	No. ESR	% ESR
ES	2 368	38.8
TP	0	0.0
RA	2 154	35.3
FO	131	2.1
WE	1 094	17.9
QS	120	2.0
MS	237	3.9
Total	6 104	100

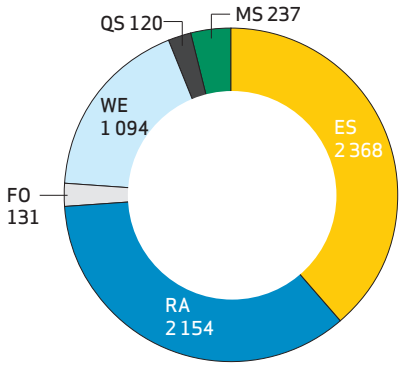


Figure 10.1: Short-term toxicity to fish (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

ESRs, registrants chose weight of evidence. (Q)SAR predictions were reported in 58 ESRs, which is half the amount of those (Q)SAR predictions found in the short-term toxicity to fish entries.

For the Annex X dossiers, 4 041 ESR entries were identified (760 more than in the previous data pool in 2011). Registrants substantially confirmed the choices of 2011, with a small increase in the use of experimental studies (2.3%) and weight of evidence (2.9%), while displaying a minor reduction in the testing proposals (0.3%) and (Q)SAR studies (0.1%). The choice of experimental studies has decreased by 4.1%, whereas read-across maintained the same ratio as in 2011.

Similarly, the percentage of experimental studies for the non-phase-in substances at or above 100 tonnes per year remained stable (14.2% of ESRs). While the most frequent option chosen by registrants was still to propose to omit the study (63.1% of the cases), the figure has decreased by 2.2% compared to the previous data pool. Contrarily, an increase of 4.7% has been identified for weight of evidence and an increase of 5.9% for read-across. Detailed results including relative differences between both data pools are provided in Table 5 of the main text.

Long-term toxicity to fish (ENV)		
	No. ESR	% ESR
ES	420	11.8
TP	25	0.7
RA	1 025	28.8
FO	1 515	42.5
WE	462	13.0
QS	58	1.6
MS	58	1.6
Total	3 563	100

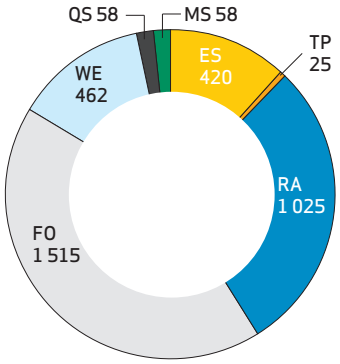


Figure 10.2: Long-term toxicity (fish) (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 11: Long-term or reproductive toxicity to birds

Information on long-term or reproductive avian toxicity needs to be considered only for substances manufactured or imported in quantities of at least 1 000 tonnes per year (i.e. an Annex X requirement).

The data may be needed to assess the secondary poisoning risks to predators following chronic exposure to a substance through the fish and earthworm food chains. Given that mammalian toxicity is considered in detail for human health protection, the need for additional data for birds must be considered very carefully – new tests are a last resort in the data collection process. However, birds are fundamentally different from mammals in certain aspects of their physiology (e.g. the control of sexual differentiation, egg laying, etc.), and so mammalian toxicity data are of limited predictive value for birds.

The need to conduct a secondary poisoning assessment is triggered by a number of factors. If these criteria are not met, then further investigation of chronic avian toxicity is unnecessary. However, if the substance has a bioaccumulation potential and a low degradability (for example, it is not readily biodegradable or not hydrolysable) and also has a potential to cause toxic effects if accumulated in higher organisms, a detailed assessment of secondary poisoning should be conducted.

Avian toxicity tests are often carried out for substances with intentional biological activity as a result of other regulatory approval requirements (especially active substances used in plant protection products, veterinary medicines and in biocides). They are rarely performed for most other substances. When available from other regulatory approval requirements, such data are relevant for REACH purposes as a source of analogue data or when the substance also has other uses that need to be registered under REACH. In addition, avian toxicity data may be considered on a case-by-case basis in the assessment of toxicity for PBT assessment but avian toxicity data will not only be necessary for this purpose alone.

No specific avian *in vitro* methods are currently available or under development.

Presented below are the data on the ESRs for this endpoint. In addition, trends are considered, where relevant, by reference to Table 5, i.e. the difference in the relative proportion of ESRs (by IUCLID purpose flag), found in 2011 versus 2013.

As presented in Figure 11.1, for phase-in substances between 100 and 1 000 tonnes per year for this endpoint, ECHA has found 975 ESR entries. Only 123 of the ESRs contained experimental studies (12.6% of all ESR entries

for this endpoint). In just over half of the cases (51.7%), registrants proposed to omit the study. A total of 198 entries contained data on read-across (20.3%) and 132 on the weight of evidence approach (13.5% of the ESRs).

For Annex X dossiers, 2 435 ESR entries were identified, i.e. an increase of 428 entries compared to the first data pool. The distribution of registrants' selections has been very similar to that of the previous submission, with variations of less than 1% for experimental studies, read-across and (Q)SAR. The most used option has again been a possibility to omit the study (67.2%, corresponding to 1 637 entries); however, this was 5.5% less than in the previous data pool. In proportion, weight of evidence was used more than in the previous submission (2.8% relative increase).

For the non-phase-in substances at or above 100 tonnes per year, only 104 ESRs have been submitted and 77.9% contained choices to omit the study. However, it has to be noted that this figure represents a 13.8% reduction compared to data reported in 2011; whereas a 12.5% increase (13 new entries) have been identified as experimental studies and 5.9% used the weight of evidence to cover this endpoint. Detailed results including relative differences between both data pools are provided in Table 5 of the main text.

Long-term toxicity to birds (ENV)		
	No. ESR	% ESR
ES	123	12.6
TP	0	0.0
RA	198	20.3
FO	504	51.7
WE	132	13.5
QS	1	0.1
MS	17	1.7
Total	975	100

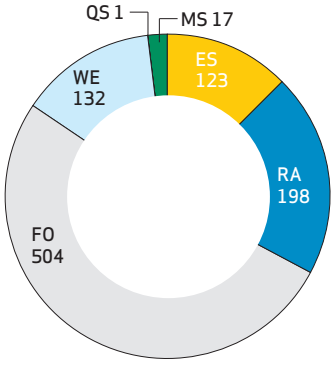


Figure 11.1: Long-term toxicity (birds) (1 882 dossiers covering phase-in substances 100-1 000 tonnes per year, one or more ESRs may be present per dossier)

Legend
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Appendix 12: Analysis of adaptations made according to Annex XI of the REACH Regulation

As noted in section 3.4 of the report, this appendix provides the results from analyses of how registrants used the adaptation possibilities of Annex XI to the REACH Regulation. The adaptations analysed in this section were the use of read-across and categories, weight of evidence and (Q)SARs.

12.1 GROUPING OF SUBSTANCES AND READ-ACROSS

Details on the methodology used and a summary of the overall outcomes are provided in section 3.4 of the report.

As outlined in the latter section, the use of read-across was analysed from two perspectives. The first projection of the data concerned the use of read-across submitted in registrations without using the IUCLID category template. Secondly, the use of read-across or grouping instances submitted using the IUCLID category template was analysed. The IUCLID category template allows registrants to link together registrations from different substances intended by the registrant to be considered within a grouping and read-across approach and facilitates the automatic generation of a data matrix. However, the IUCLID standard template also accommodates the submission of grouping and read-across approaches.

The data pool for the first projection was the same as for the ESR approach. ECHA analysed 3 813 lead and individual dossiers covering 3 662 substances at or above 100 tonnes per year (Table 2 of the section 3.1 of this report). For the second projection, only those dossiers submitted using the IUCLID category template, and therefore excluded from the main data pool, were analysed. This second pool consisted of 649 IUCLID category dossiers (533 lead and individual dossiers, 116 member dossiers) covering 523 substances at or above 100 tonnes per year, representing 121 different categories, as defined by the registrants. There were 26 cases where the same substance was covered by category and non-category dossiers, therefore the total number of substances covered in the combined pool was 4 159.

As outlined in Appendix 13 of this report, read-across is a technique for filling data gaps using either the analogue and category approach. However, for the purpose of this report, the extracted read-across cases for both the analogue and category approach (without using the IUCLID template) are combined, while the read-across reported by the registrants within the IUCLID category template is considered separately. The results of the two approaches are compared below.

12.1.1 First projection: the use of read-across without the IUCLID category template

ESRs with the IUCLID study result type "read-across based on grouping of substances" and "read-across from supporting substance" as specified by the registrant in the "study result type" are counted cumulatively. In addition, ESRs with the study result type "experimental study", where the registrant has indicated that the test substance is "different than the registered substance" were also counted as read-across cases in this analysis. More than one read-across study can be provided for a given endpoint and substance. Irrespective of how many read-across ESR there were for a given endpoint and substance, each substance was counted only once.

The total number of ESRs with read-across identified according to the criteria described above is 53 171. The number of substances per endpoint containing at least one read-across endpoint study record is shown in Figure 12.1. The transformation of the number into a number of substances was achieved by identifying substances by EC number, for which the dossiers contained at least one ESR with read-across. The total number lead to a total of 2652 substances. Genetic toxicity *in vitro* was excluded from all analyses in this appendix since this endpoint does not require tests performed with vertebrate animals.

As presented in Figure 12.1, read-across was most frequently used for repeated dose toxicity combining all routes (2 074 substances). In order of decreasing frequency, read-across was used for the endpoints "reproductive toxicity" (1 613 substances), "developmental toxicity/teratogenicity" (1 519 substances), and acute toxicity combining all routes (1 577 substances). For the environmental endpoints, read-across was used most frequently to fulfil information requirements for acute toxicity to fish (1 505 substances). Least frequently this approach was used for the endpoints which do not fall under standard information requirements under REACH: "carcinogenicity" (639 substances) and "toxicity to birds" (553 substances).

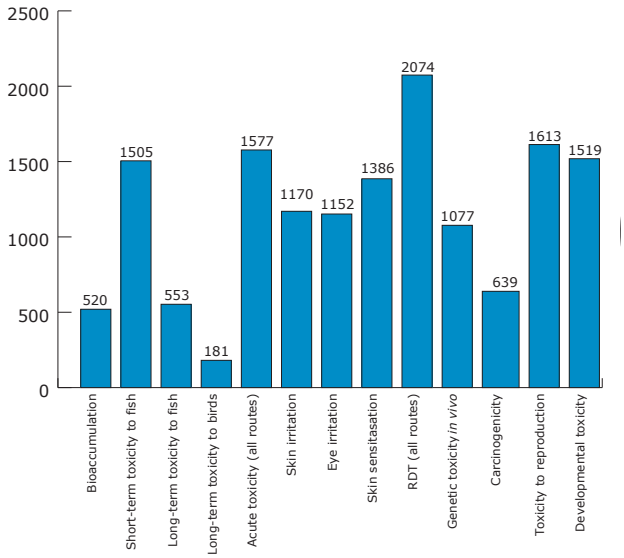


Figure 12.1: Number of substances with identified read-across ESRs not in category template by endpoint

The data were also analysed by the purpose flag selected by the registrant. Guidance on how to set purpose flags is available in "Data Submission Manual Part 05 - How to complete a technical dossier for registrations and PPORD notifications". The distribution of these ESRs per endpoint and the purpose flag[1] are shown in Table 12.1. It should be noted that for one substance there may be more than one ESR containing read-across for a given endpoint. The sums of the ESRs for 13 endpoints are expressed as totals. The proportion of ESRs per endpoint totals 100% across each endpoint row. In the columns, an average percentage is shown as an aggregation of the results per purpose flag for different endpoints. It should be noted that the average percentage has been calculated as a simple average of percentages (within analysed endpoints). The overall average does not equal the ratio between the total numbers of ESRs.

1 http://echa.europa.eu/documents/10162/13653/dsm5_tech_dossier_en.pdf
Data Submission Manual Part 05 - How to complete a technical dossier for registrations and PPORD notifications

Table 12.1: Distribution of ESR with read-across per purpose flag and per endpoint

Endpoint	ESR	Key Study	Supp. Study	WoE	Not Assigned				
	count	count	%	count	%	count	%	count	%
Bio accumulation	1 001	417	42	225	22	350	35	9	1
Short-term toxicity to fish	7 155	1 823	25	2 884	40	1 873	26	575	8
Long-term toxicity to fish	3 057	1 342	44	1 073	35	315	10	327	11
Long-term toxicity to birds	875	221	25	235	27	397	45	22	3
Acute toxicity (all routes)	8 001	3 346	42	3 091	39	1 289	16	275	3
Skin irritation	3 927	1 458	37	1 608	41	721	18	140	4
Eye irritation	3 202	1 314	41	1 238	39	494	15	156	5
Skin sensitisation	3 359	1 433	43	950	28	906	27	70	2
RDT (all routes)	9 593	3 145	33	4 293	45	1 874	20	281	3
Genetic toxicity *in vivo*	2 873	1 328	46	854	30	571	20	120	4
Carcinogenicity	2 332	882	38	1 010	43	376	16	64	3
Toxicity to reproduction	3 576	1 737	49	803	22	971	27	65	2
Developmental toxicity	4 220	1 834	43	1 293	31	954	23	139	3
Total	53 171	20 280		19 557		11 091		2 243	
Average			39		34		23		4

12.1.2 Second projection: the use of read-across in the IUCLID category template

This part of the analysis aims to gain a better understanding on how the category approach was used by registrants and provides an indication of how data rich the categories may be. The data are taken from dossiers where the category template of IUCLID was used. In this analysis, both Annex IX (> 100 tpa) and X (> 1000 tpa) substances are included.

The total number of ESRs analysed in the category template dossiers is 57 370 (covering 517 substances). In analysing data submitted in the IUCLID category template, it has to be noted that registrants often use one general template with many studies aiming to cover the whole category (and not only one member of this category) in all category dossiers. As a consequence, the same set of studies is copied to all dossiers of all category members. Therefore, category dossiers appear to contain many more ESRs than non-category ones.

A plot of the number of substances with at least one endpoint filled by category template is shown in Figure 12.2.

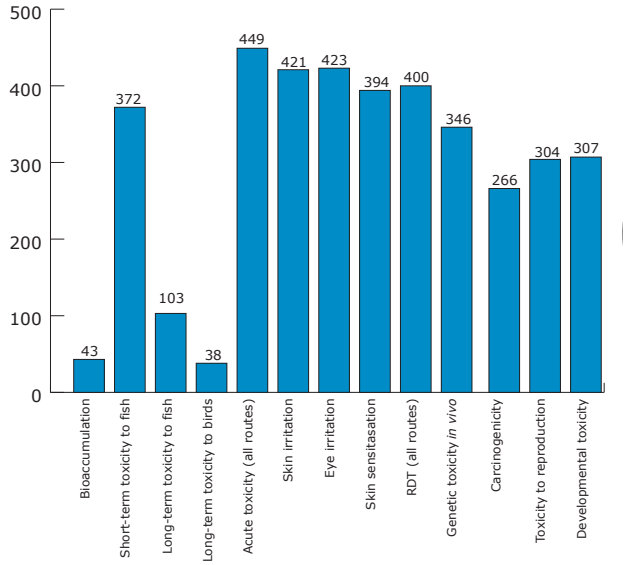


Figure 12.2: Number of substances with identified use of category template by endpoint

As can be seen in Figure 12.2, the distribution of read-across in the dossiers of category template differs slightly for human health data, if compared to Figure 12.1, but demonstrates a similar trend for environmental endpoints. In the dossiers built using the category template, the read-across approach was most frequently used for "acute toxicity (all routes)" – in 449 substances, shortly followed by eye and skin irritation (423 and 421 substances, respectively) and skin sensitisation (394 substances). For environmental endpoints, read-across was most frequently used for acute toxicity to fish (372 substances). Read-across has been used least frequently for the endpoints "bioaccumulation" (43 substances) and "toxicity to birds" (38 substances).

Due to the difficulties in distinguishing which substance is the target and which is the source in category template dossier(s), a projection based on purpose flag was not conducted.

12.1.3 Avoiding redundancy in the read-across analysis

To assess the use of read-across (with and without the IUCLID category template), the unique experimental study (UES) concept has been applied. The assumption is that the availability of a unique experimental study on one substance is used to substitute an endpoint study record (by read-across) in another substance. This means that the study content has been analysed to identify the unique ESRs in the dossier pool and to avoid redundant counting of unique experimental studies if they have been used in several ESRs. More details of the methodology are provided in section 3.4 of this report.

The results of the unique experimental study analysis are presented in Table 12.2

ECHA acknowledges the fact that some studies could provide information for two or more endpoints. For the statistical purposes of this report and to avoid the double counting of studies that cover more than one endpoint, unique studies and corresponding ESRs were counted only once, under the IUCLID section number where they were identified for the first time by the algorithm. The OECD 422 (combined repeat dose toxicity and toxicity to reproduction screening study) and its guideline equivalents are,

Table 12.2: ESR substitution ratios for category and non-category template dossiers

IUCLID Section	CAT	CAT	CAT	Non-CAT	Non-CAT	Non-CAT
	No of ESRs	No of UESs	ESR/UES	No of ESRs	No of UESs	ESR/UES
Bioaccumulation	76	22	3.5	1 377	447	3.1
Short-term toxicity to fish	2612	370	7.1	8 392	2 864	2.9
Long-term toxicity to fish	126	19	6.6	3 458	798	4.3
Long-term toxicity to birds	133	25	5.3	972	211	4.6
Acute toxicity (all routes)	16 021	1 305	12.3	9 931	4 039	2.5
Skin irritation	11 336	654	17.3	5 423	1 960	2.8
Eye irritation	5 953	476	12.5	4 038	1 605	2.5
Skin sensitization	5 423	472	11.5	3 871	1 705	2.3
RDT (all routes)	9 044	714	12.7	11 076	4 547	2.4
Genetic toxicity *in vivo*	1 932	210	9.2	3 414	1 261	2.7
Carcinogenicity	1 756	213	8.2	2 756	1 034	2.7
Toxicity to reproduction	960	137	7.0	3 986	1 745	2.3
Developmental toxicity	1 998	218	9.2	4 893	2 041	2.4
Total	57 370	4835		63 587	24 257	

according to ECHA's analysis, the greatest source of such studies. The number of UESs containing combined screening studies is 69 in category dossiers and 677 in non-category dossiers.

The ESR substitution ratio demonstrates how many ESRs are covered by one unique experimental study within an endpoint. It can be seen that the ESR substitution ratio varies greatly across the analysed endpoints. Many endpoint-specific factors (e.g. data availability, requirement for studies on multiple routes, availability of in-vitro experimental studies etc.) may influence this ratio. Therefore, the ratios at the endpoint level should not be compared and generalised.

However, by comparing the category and non-category results there is a clear trend visible for all endpoints that the ESR substitution ratio for category dossiers is significantly higher than for the non-category dossiers. This means that in category dossiers one UES covers more ESRs used by the registrants to fulfil data obligations under REACH. In addition, it must be stressed that the ESR substitution ratio does not take into account the context in which the study was reported by the registrant (key study, supporting study or weight of evidence).

12.2 THE USE OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIPS ((Q)SARS)

ESRs with the IUCLID study result types "(Q)SAR" and "estimated by calculation" (as specified by the registrant) were counted cumulatively for the purpose of the analysis (further referred to as "calculated results").

The total number of ESRs using "calculated results", counted as described above, is 3 032. The transformation of this number into a number of substances, defined by unique EC number and which contain ESR based on calculated results, leads to a total of 806 different substances. The number of substances containing at least one "calculated result" is shown in Figure 12.3 for each endpoint.

The number of these ESRs by endpoint and purpose flag are shown in Table 12.3. It should be noted that one substance may contain more than one ESR with

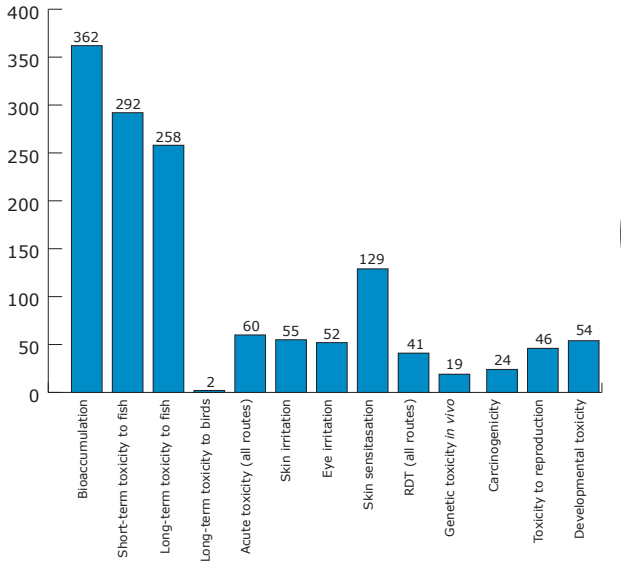


Figure 12.3: Number of substances with "calculated results" by endpoint

a "calculated result" for a given endpoint. Therefore, the proportion of ESRs per endpoint totals 100% across each endpoint row. In the columns, an average percentage as an aggregation of different endpoints per purpose flag is shown.

As can be seen in Figure 12.3, the "calculated result" methods were used most frequently for the endpoint of bioaccumulation (362 substances), followed by short-term and long-term toxicity to fish (292 and 258 substances, respectively).

12.3 THE USE OF WEIGHT OF EVIDENCE APPROACHES (WE)

ECHA identified the ESRs with the IUCLID purpose flag "weight of evidence" as specified by the registrant (and analysed only those ESRs here). Once the weight of evidence flagged ESRs were identified, the study result type of these ESRs were counted as follows:

- ESRs with study result type: "experimental results" (ES);
- ESRs with study result type: "(Q)SAR" and "estimated by calculation", merged (QS);
- ESRs with study result type: "Read-across based

Table 12.3: Number of ESRs with "calculated results" per purpose flag and per endpoint

Endpoint	ESR QS	Key Study	Supp. Study	WoE	Not Assigned				
	[count]	[count]	[%]	[count]	[%]	[count]	[%]	[count]	[%]
Bioaccumulation	1 465	495	34	451	31	510	35	9	1
Short-term toxicity to fish	568	120	21	333	59	106	19	9	2
Long-term toxicity to fish	315	162	51	62	20	80	25	11	3
Long-term toxicity to birds	2	2	100	1	0	1	0	1	0
Acute toxicity (all routes)	107	32	30	23	22	47	44	5	5
Skin irritation	72	23	32	30	42	19	26	0	0
Eye irritation	58	19	33	19	33	20	34	0	0
Skin sensitisation	165	30	18	52	32	82	50	1	1
RDT (all routes)	70	29	41	19	27	22	31	0	0
Genetic toxicity *in vivo*	34	7	21	19	56	8	24	0	0
Carcinogenicity	31	5	16	7	23	16	52	3	10
Toxicity to reproduction	72	20	28	24	33	28	39	0	0
Developmental toxicity	73	21	29	25	34	26	36	1	1
Total	3 032	965		1 065		965		40	
Average			35		32		32		2

on grouping of substances" and "read-across from supporting substance", merged (RA);
- Remaining ESRs were assigned as miscellaneous (MS). This includes empty fields and ESRs indicated as "others" in the "study result type" field, also covering IUCLID flags to omit the study (FO).

The distribution of substances containing at least one weight of evidence ESR is shown in Figure 12.4. The distribution of these ESRs per endpoint per purpose flag are shown in Table 12.4.

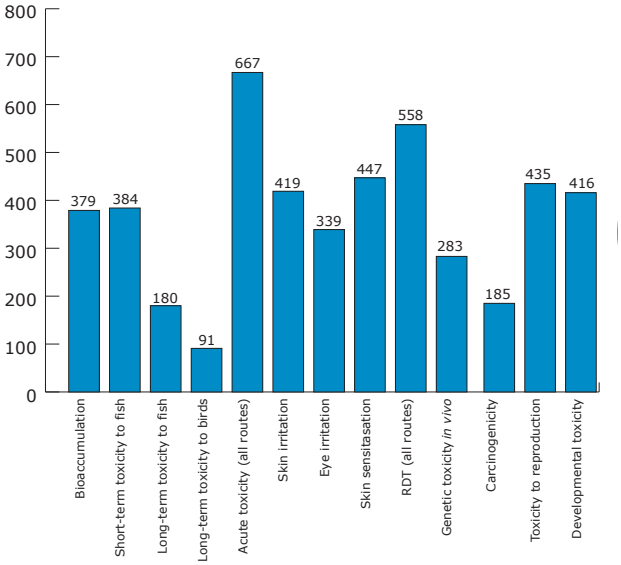


Figure 12.4: Distribution of substances containing at least one weight of evidence as the "study result type"

As shown in Figure 12.4, weight of evidence was used most frequently for the endpoints of acute toxicity, all routes (667 substances). In decreasing order, weight of evidence was found for skin sensitisation (447 substances), reproductive toxicity (435 substances), skin irritation (419 substances), and developmental toxicity/teratogenicity (416 substances). Less frequently, weight of evidence was used for carcinogenicity (185 substances) and toxicity to birds (91 substances) mirroring the situation for read-across.

The total number of ESRs flagged by the registrant as weight of evidence is 18 636. The transformation of this number into ESRs per substance, defined as a unique EC number, showed that a total of 1 856 substances contain at least one ESR flagged as weight of evidence.

Notably, one substance may contain more than one ESR with weight of evidence for a given endpoint. Therefore, the proportion of ESRs per endpoint totals 100% across the endpoint rows. In the columns, an average percentage as an aggregation of different adaptation per endpoints is shown.

It is interesting to note that on average, most of the weight of evidence flagged ESRs were filled with read-across (50%), followed by experimental data (41%) (Table 12.4). The proportion of use of (Q)SARs was significantly lower (5%).

12.4 AGGREGATION OF ENDPOINTS PER SUBSTANCE

The aggregation of ESRs per substance expressed as a unique EC number are given in Figures 12.1, 12.2, 12.3 and 12.4. The corresponding numbers are shown in Table 12.5.

Since there were often several adaptations found for different endpoints in one substance, the number of unique substances affected (based on unique EC numbers) was counted separately per adaptation (and is not deducible from Table 12.5). Thus, read-across (without the IUCLID category template) was used in a total of 2 652 unique substances. Weight of evidence was used for a total of 1 856 substances. (Q)SARs were used in a total of 806 unique substances. Read-across in the IUCLID category template was used in a total of 517 unique substances, affected, i.e. those of the scope of the analysis performed for this appendix.

Table 12.4: Distribution of ESR with weight of evidence per study result type and per endpoint

Endpoint	ESR WE	ESR ES	ESR RA	ESR QS	ESR MS				
	[count]	[count]	[%]	[count]	[%]	[count]	[%]	[count]	[%]
Bioaccumulation	1 437	565	39	234	16	510	35	128	9
Short-term toxicity to fish	2 467	1 088	44	1 217	49	106	4	56	2
Long-term toxicity to fish	966	501	52	274	28	80	8	111	11
Long-term toxicity to birds	450	66	15	365	81	0	0	19	4
Acute toxicity (all routes)	2 893	1 507	52	1 262	44	47	2	77	3
Skin irritation	1 284	705	55	549	43	19	1	11	1
Eye irritation	897	513	57	353	39	20	2	11	1
Skin sensitization	1 374	445	32	816	59	82	6	31	2
RDT (all routes)	2 491	809	32	1 581	63	22	1	79	3
Genetic toxicity *in vivo*	1 006	501	50	476	47	8	1	21	2
Carcinogenicity	728	314	43	337	46	16	2	61	8
Toxicity to reproduction	1 256	365	29	829	66	28	2	34	3
Developmental toxicity	1 387	368	27	956	69	26	2	37	3
Total	18 636	7 747		9 249		964		676	
Average			41		50		5		4

Table 12.5: Distribution of substances per adaptation and per endpoint.

Endpoint	Number of substances (Categories)	Number of substances (RA)	Number of substances (QS)	Number of substances (WE)
Bioaccumulation	43	520	362	379
Short-term toxicity to fish	372	1 505	292	384
Long-term toxicity to fish	103	553	258	180
Long-term toxicity to birds	38	181	2	91
Acute toxicity (all routes)	449	1 577	60	667
Skin irritation	421	1 170	55	419
Eye irritation	423	1 152	52	339
Skin sensitisation	394	1 386	129	447
RDT (all routes)	400	2 074	41	558
Genetic toxicity *in vivo*	346	1 077	19	283
Carcinogenicity	266	639	24	185
Toxicity to reproduction	304	1 613	46	435
Developmental toxicity	307	1 519	54	416

Appendix 13: Alternative test methods and approaches: developments

This appendix summarises recent developments impacting various alternative test methods and approaches and aims to guide registrants through their potential regulatory use. Several newly adopted test guidelines became available before publication of this report; a detailed analysis of how registrants made use of new test methods is provided in appendices 1 to 4.

Currently, a number of *in vitro* test methods have either been recently validated or are under validation for acute toxicity, skin sensitisation, eye irritation, and for fish toxicity. These are detailed below.

13.1 OVERVIEW OF *IN VITRO* METHODS: RECENTLY VALIDATED OR UNDERGOING VALIDATION

Acute toxicity (REACH Annex VII)
The *in vitro* cytotoxicity test (3T3 Neutral Red Uptake) has been validated by the European Reference Laboratory for Alternative Methods (EURL ECVAM) for identifying substances with acute oral LD50 >2 000 mg/kg b.w. The EURL ECVAM Recommendation has been subject to public consultation and the final recommendation was published in May 2013[1]. Hence, the 3T3 Neutral Red Uptake test may have an application in weight of evidence approaches under the REACH Regulation (Annex XI; paragraph 1.2).

Skin sensitisation (REACH Annex VII)
Based on the Adverse Outcome Pathway (AOP) document published by the OECD[2], a number of approaches integrating information from *in silico*, *in chemico* and *in vitro* methods including the validated DPRA, KeratinoSensTM and h-CLAT test methods are being proposed for skin sensitisation hazard assessment purposes.

The implementation of the AOP paper is a priority for ECHA with a view to the 2018 registration deadline. Skin sensitisation is an Annex VII information requirement, which needs to be addressed by the affected registrants.

The EURL ECVAM recommendations for two test methods have been published. The EURL ECVAM recommendation for the DPRA assay was published in December 2013[3] and its recommendation for

1 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/3t3-nru-recommendation

2 http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)10/part1&doclanguage=en

3 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/eurl-ecvam-recommendation-on-the-direct-peptide-reactivity-assay-dpra

the KeratinoSensTM assay in February 2014[4]. Test Guidelines for the DPRA and the KeratinoSensTM test methods are currently being developed within the OECD test guideline programme.

Fish toxicity (potentially REACH Annex IX)
At the moment, there are relatively few test methods that could help to reduce the number of fish used for testing for REACH purposes. The Fish Embryo Toxicity (FET) Test was officially approved as an OECD Test Guideline (OECD 236[5]) in July 2013 (no corresponding EU Test Method is currently available). The applicability of the test for REACH registration purposes is now under consideration.

13.2 EYE AND SKIN IRRITATION AND CORROSION: NEW TEST GUIDELINES

Since the first report was published in June 2011, the existing *in vitro* test guidelines have been updated. In addition, new *in vitro* test guidelines introduced to assess skin and eye irritation and corrosion have been published. These test guidelines are relevant for dossiers falling under REACH Annex VII (requirement for *in vitro* studies) and potentially for Annex VIII information requirements (requirement for *in vivo* studies, but adaptations may apply).

Regarding skin irritation and corrosion, the OECD revised three test guidelines in 2013: the transcutaneous electrical resistance test (TER) for assessing skin corrosion, EU B.40, OECD 430[6]; the Human skin model test for assessing skin corrosion (includes more than one test method), EU B.40 bis, OECD 431[7]; and one skin irritation test guideline, the reconstructed human epidermis test (which also includes more than one test method), EU B.46, OECD 439[8].

The OECD 430 revision now contains performance standards that can be used for the assessment of other similar and modified TER-based test methods. The OECD 431 revision contains the inclusion of sub-category determination, i.e. corrosives category 1A and 1B/C. This test guideline, however, does not allow distinction between sub-categories 1B and 1C. The inclusion of the sub-category determination may be helpful for assigning a suitable packing group for transport purposes.

In addition, the revised OECD 431 test guideline also now contains performance standards that can be used for the assessment of other similar and modified RhE-based test methods. Moreover, it now contains instructions on how to address chemicals that directly reduce the chemical dye used in the cell viability assessment or that possess a colour directly interfering with the measurement of the reduced dye by the tissue (to avoid obtaining incorrect results from the test). Finally, it includes two new test methods – the SkinEthic™ RHE and the epiCS®. The revised test guideline for skin irritation (OECD 439) in 2013 now includes a new test method – LabCyte EPI-MODEL24 SIT. The validation of the LabCyte EPI-MODEL24 SIT method was based on the performance standards specified in Annex 4 to the OECD 439 test guideline.

A new test guideline for the assessment of serious eye damage was published in October 2012: the Fluorescein leakage test method (OECD TG 460[9]). This is an *in vitro* assay that may be used to identify water-soluble chemicals inducing serious eye damage as defined in the CLP Regulation as Category 1. While this test method is not considered valid for use as a complete replacement of the *in vivo* rabbit eye (Draize) test, it is however recommended by the OECD to be used as part of a tiered testing strategy for the regulatory classification and labelling of chemicals. Thus, this method is recommended to identify chemicals inducing

4 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/file-kerati/JRC_SPR_Keratinosens_Rec_17_02_2014.pdf

5 http://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en

6 http://www.oecd-ilibrary.org/environment/test-no-430-in-vitro-skin-corrosion-transcutaneous-electrical-resistance-test-method-ter_9789264203808-en

7 http://www.oecd-ilibrary.org/environment/test-no-431-in-vitro-skin-corrosion-reconstructed-human-epidermis-rhe-test-method_9789264203822-en

8 http://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method_9789264203884-en

9 http://www.oecd-ilibrary.org/environment/test-no-460-fluorescein-leakage-test-method-for-identifying-ocular-corrosives-and-severe-irritants_9789264185401-en

serious eye damage, specifically for limited types of chemicals (i.e., water-soluble substances and mixtures).

In addition, the Bovine Corneal Opacity (BCOP) OECD test guideline (TG 437[10]) and the Isolated Chicken Eye (ICE) OECD test guideline (TG 438[11]) were revised in 2013. The revision allows for the identification of substances, within the test methods applicability domain, not requiring classification (no category) for eye irritation in addition to the identification of chemicals inducing serious eye damage falling under Category 1 as defined by the CLP Regulation.

Regarding eye irritation, there are no test methods available or currently under validation for the detection of Category 2 eye irritants as defined by the CLP Regulation, hence *in vivo* testing may be needed to fully cover information requirements for this endpoint.

ECHA recommends registrants who are contemplating new studies for the purposes of registration in 2018 to keep up-to-date with new test methods as these may be useful in avoiding unnecessary animal testing. Especially in relation to eye irritation, the updated test guidelines for BCOP and ICE published by the OECD will allow more broad assessment of the potential effects on the eye from the hazard and classification and labelling perspective and may help to reduce further testing on animals.

ECHA therefore launched a dedicated page on its website, providing information on new and revised test guidelines, and advising on their use for REACH purposes[12].

10 http://www.oecd-ilibrary.org/environment/test-no-437-bovine-corneal-opacity-and-permeability-test-method-for-identifying-i-chemicals-inducing-serious-eye-damage-and-ii-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_9789264203846-en

11 http://www.oecd-ilibrary.org/environment/test-no-438-isolated-chicken-eye-test-method-for-identifying-i-chemicals-inducing-serious-eye-damage-and-ii-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_9789264203860-en

12 http://echa.europa.eu/support/oecd-eu-test-guidelines

13.3 SKIN SENSITISATION: RECENT DEVELOPMENTS

The aspiration of ECHA and ECVAM in the area of skin sensitisation (REACH Annex VII) is to develop an integrated assessment strategy for skin sensitisation ready to be used before the 2018 REACH registration deadline for phase-in substances of 1-100 tonnes per year. The aim is that REACH registrants will be able to use an *in silico/ in chemico/in vitro* based testing strategy and prediction scheme to fulfil the REACH information requirement for the skin sensitisation endpoint.

The OECD-initiated project aims to develop a guidance document on the evaluation and application of integrated approaches to testing and assessment (IATA) for skin sensitisation. The aim of this project is to provide a generic IATA framework for skin sensitisation that would satisfy different regulatory needs (i.e. hazard assessment, hazard characterisation and risk assessment). Different IATA solutions will be described according to a consistent format, thereby providing a harmonised framework for their documentation and evaluation. In addition, the individual information sources that can be used within the IATA (e.g. physicochemical properties and alternative approaches such as (Q) SAR), *in chemico* and *in vitro* methods) will also be described using a harmonised template.

Information sources for the IATA on skin sensitisation will include the three test methods that have recently been validated: DPRA, Keratinosens™ and the h-CLAT.

The DPRA is an *in chemico* test method that addresses haptenation (i.e. the covalent binding of low molecular weight electrophilic chemicals to skin proteins), which is considered to be the molecular initiating event in the AOP for skin sensitisation. The DPRA measures peptide reactivity by quantifying, through High Pressure Liquid Chromatography (HPLC), the depletion of synthetic heptapeptides containing either cysteine or lysine following 24h incubation with the test chemical.

The Keratinosens™ is an *in vitro* test method that addresses the second key event of the skin sensitisation AOP, i.e. keratinocyte activation. The Keratinosens™ test method is a reporter gene

assay that quantifies luciferase gene induction by luminescence detection as a measure of the activation of a cyto-protective pathway in keratinocytes.

The h-CLAT is an *in vitro* test method that addresses the third key event of the skin sensitisation AOP, i.e. dendritic cell activation. This assay measures the induction of dendritic cell (DC) maturation membrane markers using Flow Cytometry in a DC-like cell line. An increase in the expression of either of these markers can be used as an indication of skin sensitisation potential.

Given the fact that these methods have a limited mechanistic coverage of the AOP for skin sensitisation, since each of them is addressing a specific key event, and considering their known limitations (i.e. lack of or limited metabolic capacity and limited applicability to test substances with poor solubility), they are proposed to be used in combination with other supporting information in a weight of evidence approach or within IATA. Nevertheless the OECD TGs which are currently being developed for DPRA and KeratinoSens™ foresee that, depending on the regulatory framework, positive results form these test methods may be used on their own to classify a test chemical into UN GHS category 1.

These developments will be further addressed in the third Article 117(3) report (due by June 2017).

13.4 (QUANTITATIVE) STRUCTURE ACTIVITY RELATIONSHIP ((Q)SAR) METHODS: NEW DEVELOPMENTS

According to REACH, non-testing methods could be used instead of results from new experimental studies to address REACH information requirements, if certain conditions are met. Currently, new biochemical and cellular assay systems and computational predictive methods are under development. The "omics" technologies are expected to provide a tool for optimising the grouping, and new high-through-put assays could indicate further similarities than those indicated by chemical structure alone. The greater biological understanding of the mode of action shifts provides new opportunities for integrating mechanistic data into (Q)SAR modelling.

Among the latest developments, the OECD QSAR Toolbox has progressed significantly. New features of the QSAR Toolbox version 3.0 include additional data sources, an advanced search engine, new mechanistically and endpoint specific profiling schemes, quantitative mixtures, tautomeric set prediction, new transformation simulators for autoxidation and hydrolysis. In version 3.1 of the QSAR Toolbox a number of these new features have been updated or extended and a new database for observed rat *in vivo* metabolism has been added. The Toolbox now incorporates, as a proof-of-concept, a new approach for estimation of skin sensitisation based on adverse outcome pathways (AOPs). The Toolbox version 3.2 was released in December 2013 and is available for free download from the QSAR Toolbox website[13]. More detailed information on the OECD QSAR Toolbox can be found in section 4.2 of this report.

According to a recent survey (Mays et al., 2012)[14], the OECD Toolbox, EpiSuite[15] and models, developed under EU funding (CAESAR[16]) are most frequently used by respondents. Notably, all three tools are freely accessible. Among the endpoints calculated using (Q)SARs, the first place is taken by the physico-chemical properties (lead by octanol-water partition coefficient – log Kow). Some user groups – such as academic users and industry consultants – also highlighted the use of (Q)SAR methods for prediction of water solubility, boiling point and vapour pressure. REACH recommends that the physico-chemical properties of the substance for registration purposes should be measured rather than predicted and the industrial stakeholders usually provide these properties as experimental values.

Structure-activity relationships (SARs) can support the identification of analogues (in this context taken to mean similar substances, for which experimental data may already be available) and assessment of their suitability for use in the building of categories and for read-across. While the selection of analogues is not considered an element of the read-across

13 http://www.qsartoolbox.org/download.html

14 Mays et al. (2012), Use and perceived benefits and barriers of (Q)SAR models for REACH

15 http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

16 http://www.caesar-project.eu/

justification, it is useful for the evaluator to know what rationale for selection of similar substances was used. ECHA always expects and highly recommends that a proper toxicological explanation of why the analogues are suitable for read-across should be provided.

The validity of applied (Q)SAR models should be assessed according to the OECD validation principles for (Q)SARs (and the criteria mentioned in Annex XI of the REACH Regulation) and the results of the analysis should be reported in detail, in a transparent way. The information needed should be in line with the headings of the (Q)SAR model reporting formats (QMRFs) and (Q)SAR prediction reporting formats (QPRFs).

It is worth saying that (Q)SAR seems more often applied for prediction of physico-chemical properties, environmental toxicity and fate parameters than for human health endpoints on the basis of the results and conclusions provided in this report (see Appendix 12 of this report).

The usefulness of different alternatives, including (Q)SARs and *in vitro* methods, could be maximised by their combination in batteries, and used also in a sequential manner to inform the need for further testing (Jaworska et al., 2011[17]; Rorije et al., 2013[18]).

13.5 READ-ACROSS APPROACH AND CATEGORIES

Under REACH, the grouping of substances and read-across is offered as a possibility for adaptation of the standard testing regime of the REACH Regulation when the conditions set in Annex XI, 1.5 are met. If the read-across approach is adequate, additional testing becomes unnecessary. A read-across approach can also support a conclusion for a REACH endpoint using a weight of evidence approach, for example, in combination with predictions from (Q)SAR methods.

The use of grouping and read-across for filling data gaps through adaptation to the standard testing regime under REACH provides a powerful tool for the reduction of the use of laboratory animals for the purpose of hazard assessment of chemical substances. However, ECHA can only accept a read-across approach when it is thoroughly justified and based on sound science, as it has to be meaningful for the purpose of hazard and risk assessment to guarantee the safe use of chemicals.

Categories and analogue approaches are both ways of grouping substances for the purpose of read-across. It is important to note that these approaches require two steps. First, the identification of structurally similar substances (grouping) and secondly the filling of a data gap by the prediction of the properties of the substance in question. The technique for data gap filling should be described, justified and clearly presented.

Conceptually, the application of grouping and read-across is closely related to (Q)SAR techniques for predicting properties for chemicals in so far as they are all based on the assumptions that the chemical structure determines the physico-chemical and the (eco) toxicological properties and fate, and that similar substances are expected to generate similar effects. (Q)SARs and read-across approaches have a common feature in that the substance of interest and the analogues associated with experimental data should be similar in some respects. Grouping of substances without obvious structural similarity is not considered possible for the filling of data gaps under REACH.

In all cases, the alternatives should:

- be scientifically mature/valid to serve the regulatory purposes for which they are proposed, and
- provide equivalent information to that obtainable from standard testing.

Critical for the application of read-across and (Q)SAR (and which also applies to *in vitro* methods) is the understanding of the domain where the estimations could be relied upon. The 'applicability domain' (or domain of application) concept is introduced to encourage the analysis of the borders, which can be used to determine the reliability and

17 Jaworska et al. (2011), Integrating non-animal test information into an adaptive testing strategy - skin sensitization proof of concept case. ALTEX. 2011;28(3):211-25.

18 Rorije et al. (2013), The OSIRIS Weight of Evidence approach: ITS for Skin Sensitisation. Regul Toxicol Pharmacol. 2013 Nov;67(2):146-56.

the uncertainty of the estimation if (Q)SAR or category-approach read-across are undertaken. Obviously, the concept of a "domain" does not apply to read-across between two analogues.

The category approach under REACH is used in combination with read-across for the purpose of data gap filling in the hazard assessment process. It should be clear that registrants cannot address categories in one registration dossier, but need to register each individual substance belonging to the category. If the category approach has been used, it is expected to result in filling a data gap to meet given information requirements. For clarification, the key differences of the historical use of categories and the requirements under REACH are:

- Shift of responsibility to the registrant to demonstrate safe use (including the responsibility to meet a tonnage-dependent and defined set of standard information requirements).
- Grouping and read-across approaches (category and analogue approaches) need to comply with the rules given in Annex XI Section 1.5 (two-step approach).
- Ownership of the source data: there is no legal obligation to share data for the purpose of read-across, but legal access to the information stemming from the analogue substances is required.

As an example of the consideration registrants may have, categories tend to be eventually reduced to those substances that are of commercial interest to a particular industry group, while other substances which may also support the category are omitted.

ECHA has developed an illustrative example of a grouping of substances and read-across approach to support companies to comply with their obligations under REACH[19]. The illustrative example includes several elements, such as an introductory note which provides background information on read-across including general considerations and addresses shortcomings commonly identified by ECHA when evaluating registration dossiers, and an example, which contains an illustrative example for a hypothetical substance intended to outline the level of information expected to be provided and includes explanatory comments which expand on the reasoning and approach taken. Additional illustrative examples will be provided in the future.

A clear description of the identities of both the source and the target substances is required to demonstrate the structural relationships between source and target substances. For the purpose of substantiating the relationship between the source and the target substances, this description may need to go well beyond the level of detail required by Annex VI for compliance of the dossier. The registrant must have access to the data used in a registration dossier including such data used for read-across.

Read-across may be supported by other relevant and available data such as (Q)SAR results e.g. in a weight of evidence approach to reduce uncertainty. The increased uncertainty of the prediction of a property must be accounted for when using this prediction for derivation of DNEL or PNEC values.

Currently, read-across is widely used by REACH registrants. Such non-standard information has to be equivalent to the information obtained from the standard studies, in that the key parameters of the standard method should be addressed and the result must be suitable for adequate risk assessment and/or classification. Therefore, registrants have to justify these adaptations of the standard testing regime in the registration dossier by providing scientific explanations and experimental evidence, where necessary and applicable.

In particular, ECHA would like to stress, that a noticeable improvement in the registrants' approach and efforts on how to use read-across has been made. More specifically, ECHA together with the Member States conditionally accepted several large category and read-across approaches (as proposed by registrants). A more detailed outcome is provided in section 2.5 of this report.

13.6 ITS AND WEIGHT OF EVIDENCE APPROACHES

An integrated testing strategy (ITS) is generally defined as an approach that combines one or more

19 http://echa.europa.eu/en/support/grouping-of-substances-and-read-across

non-animal approaches, possibly with animal studies, to fulfil the information requirements, covering all aspects of a specific endpoint. For lower-tier endpoints such as skin and eye irritation and skin sensitisation, the approach may be a clear step-wise approach or a test battery including various non-animal approaches. Under the umbrella of the OECD, the regulatory agencies have initiated a long-term project on integrated approaches to testing and assessment (IATA). In some cases, only non-animal approaches could then be potentially used in a weight of evidence approach or as a standard information requirement in REACH. For skin and eye irritation, the ECHA guidance describes a tiered approach as new methods have been developed. The development of an IATAs for skin irritation/corrosion and skin sensitisation by the OECD is underway. The development of IATA for skin irritation/corrosion is in its final stages and is expected to be published in the near future. The development of IATA for skin sensitisation has only recently started and will still take some time before it is finalised.

For higher-tier human health endpoints, such as repeated dose toxicity and reproductive toxicity, no comprehensive ITS or IATA exists or is foreseen to be available in the near future. In fact, the incremental implementation of information requirements under REACH (i.e. at Annex VIII, the requirements of Annex VII must also be met), depending on the tonnage band of the registrant, can be seen as an ITS.

The information requirements themselves for repeated dose toxicity and reproductive toxicity include animal studies from screening to definitive studies across increasing tonnage level. However, on a case-by-case basis, the weight of evidence available from several independent sources of information may be used to assess whether the evidence is sufficient to assume/conclude that a substance has a particular dangerous property or not (Annex XI, 1.2). Specific information requirements in the REACH Annexes may then be adapted or fulfilled. These adaptations can include validated and relevant non-animal approaches, read-across approaches and other sources of information subject to adequate risk assessment and risk management measures being in place.

Studies on repeated dose toxicity and reproductive toxicity address a huge variety of parameters, many of which are interrelated and, thus, are challenging to break into meaningful pieces (key elements) for the development of ITS/IATA.

Basic elements of an ITS/IATA are, for example, physicochemical data, *in vitro* data, human data, animal data, computational methods, "omics" data, mechanisms/modes of action (MoA) and biokinetic models. These basic elements may either cover single parameters (events) of the information requirement (e.g. toxicity to developing follicles in reproductive toxicity) or may be combined in various ways to cover one or more parameters for a REACH information requirement. Basic elements may be combined in tiered testing strategies, test batteries, read-across, category building, weight of evidence, or adverse outcome pathways.

At present, a number of non-animal tests are in different stages of development, but there is not a single non-animal approach nor a generally accepted combination of non-animal approaches to cover all parameters for any given human health higher tier endpoints. It seems most likely that each substance will require its own substance and case-specific testing approach, which has to be evaluated individually and scientifically, taking into account the multiplied uncertainties arising from a combination of numerous elements. ECHA currently keeps track of the development of new methods and approaches and their combinations to evaluate their potential applicability under REACH. In the context of REACH, any approach must produce information usable for a robust risk assessment and/or for classification and labelling.

13.7 OTHER REGULATORY DEVELOPMENTS

This section gives a general overview of the roles and responsibilities of different parties involved in the development and acceptance of alternative approaches to animal testing. This section does not cover other activities related to the development, validation and assessment of alternative methods in which national or international bodies are involved.

European Commission

As highlighted in the first ECHA report, the protection and welfare of animals is an area covered by a wide range of EU legislation. The conduct of

studies on animals, whether for the development or production of new medicines, for studying physiological or environmental effects, or for the testing of chemical substances or new food additives, has to be carried out in compliance with EU legislation.

On 22 September 2010, the EU adopted Directive 2010/63/EC[20] to update Directive 86/609/EEC[21] on the protection of animals used for scientific purposes. The aim of the new Directive is to strengthen the legislation, and improve the welfare of those animals whose use in experimental procedures is still necessary, as well as and to firmly anchor the principle of the 3Rs in EU legislation. This Directive took full effect from 1 January 2011. In March 2013, the European Commission established an Expert Working Group for Project Evaluation and Retrospective Assessment of projects to facilitate the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. All Member States and main stakeholder organisations were invited to nominate experts to participate in the work. The main objectives of this group were to develop guidance and principles for project evaluation and retrospective assessment in line with Articles 38 and 39 of the Directive to assist all those involved in the preparation, evaluation and assessment of projects.

Newly-developed alternative test methods are validated in order to assess their relevance and reliability before they can be considered to have regulatory acceptance. In 1991, the Commission set up the European Centre for the Validation of Alternative Methods[22] (ECVAM) to promote the validation of alternative test methods including the dissemination of information about the development of advanced and alternative methods. ECVAM is part of the Institute for Health and Consumer Protection (IHCP) of Directorate General Joint Research Centre (DG JRC) of the European Commission. One of the main tasks of ECVAM is to validate alternative test methods that replace, reduce and refine the use of animals in scientific procedures and, consequently, to reduce animal experiments in the EU. Due to the increasing need for new test methods to be developed and proposed for validation in the EU, in 2011 ECVAM formally became the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM).

The Test Methods Regulation (Commission Regulation (EC) No 440/2008[23]) governs the conduct of testing for the REACH Regulation. Prior to changes in the Test Method Regulation, the regulatory acceptance of a method in the EU has to be ensured. The Commission has a responsibility, having consulted stakeholders, to propose changes to the Test Methods Regulation. It was recognised that there was a need to streamline the procedures relating to the regulatory acceptance of validated alternatives to animal testing. The Commission committed itself to improving the acceptance process by introducing a mechanism of "preliminary analysis of regulatory relevance" (PARERE) to be established (see below). The consultation networks involve EU Member State contact points and relevant agencies and committees, including ECHA. To expedite the process of regulatory acceptance of alternative test methods, it was considered that regulators should be involved as early as possible in providing a preliminary view on the potential regulatory relevance of methods submitted to ECVAM for validation.

The Commission also collects and publishes statistics on the use of animals used for experimental procedures. The latest report[24] (the seventh, published in December 2013) provides statistics from 2011. It is noteworthy that, as outlined in the seventh report, the total number of animals used for experimental and other scientific purposes in 2011 (with one Member State reporting for 2010) decreased to just below 11.5 million. This is a reduction of over half a million animals used in the EU from the number reported in 2008.

The revised EU Directive on the protection of animals used for scientific purposes (2010/63/EU) states that "Member States shall nominate a single point of contact to provide advice on the regulatory relevance and suitability of alternative

20 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

21 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:en:HTML

22 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam

23 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0001:EN:PDF

24 http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

approaches proposed for validation". Following an invitation from the Commission to nominate such single points of contact, Member States have nominated representatives to form the network of Member States. In addition, it was also considered important to involve the EU regulatory agencies, and requests for nominations from the European Food Safety Authority (EFSA), ECHA, and the European Medicines Agency (EMA) were also made. Together, they constitute the Preliminary Assessment of Regulatory Relevance network, known as PARERE[25].

PARERE started its work in 2011. The main roles of PARERE are:

- Upstreaming input on potential regulatory relevance and the suitability of proposed test methods and testing strategies.
- Facilitating information flow between EURL ECVAM and regulators regarding the development and validation of methods and identifying areas that need specific attention.
- Identifying regulatory experts to participate in specific EURL ECVAM activities (e.g. Validation Management Groups, expert workshops …)
- Commenting on EURL ECVAM strategy documents in the various toxicological areas.
- Commenting on draft EURL ECVAM Recommendations following ESAC Peer Review of validation studies.
- Supporting and promoting the role of EU- NETVAL laboratories in Member States to facilitate their participation as a testing and/or lead laboratory in EURL ECVAM led validation studies.

More information on PARERE and its activities is available at the website of EURL ECVAM.

Dissemination of information about advanced and alternative methods

A first step in meeting REACH information requirements[26] is to make full use of existing information. Therefore, readily access to suitable and adequately described (non-animal) methods is a prerequisite for their use within decision making processes by regulators for safety assessments. The EURL ECVAM is managing information systems providing reviewed and factual information on advanced and alternative methods for toxicological assessments and related fields of sciences that should be considered during information gap analysis or in a weight of evidence approach for REACH registration purposes:

i) ***In vitro*** **methods**

- The DataBase service on ALternative Methods to animal experimentation (DB-ALM[27]) provides useful information on various aspects of *in vitro* methods. The database contains in first place comprehensive and evaluated descriptions of methods that are in use or under development as well as those validated and/or accepted for regulatory purposes together with information on their applicability and related data.
- The TSAR[28] (Tracking System for Alternative test methods towards Regulatory acceptance) is another source of information to be considered when deciding whether *in vitro* tests could provide the information needed for data gap filling. TSAR has been set up to track progress, in a transparent manner, from proposal of an alternative method for validation through to its final adoption by its inclusion into the regulatory framework (EU, OECD and related)

ii) (Q)SAR models

- The (Q)SAR Model database[29] provides structured and peer-reviewed information on key characteristics of (Q)SAR Models for physicochemical, environmental and human health effects.

Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)

In 2009, the Health Programme of DG Research defined a long-term target, the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT[30]). This is an EU initiative on alternative methods to animal testing, such as *in vitro* and computational prediction

25 http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/scientific-advice-stakeholders-networks/parere

26 http://echa.europa.eu/support/information-toolkit; http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

27 Access: http://ecvam-dbalm.jrc.ec.europa.eu

28 Access: http://tsar.jrc.ec.europa.eu/

29 Access: http://qsardb.jrc.it/qmrf/

30 http://www.seurat-1.eu/

methods, to be used as a basis for an improved and more informative safety assessment of chemicals focusing on health effects after repeated exposure. SEURAT-1 is a EUR 50 million five-year research programme that started in 2011, co-sponsored by the European Commission (FP7) and Cosmetics Europe, the industrial personal care association. It is matching the U.S.Tox21 program[31], which is a U.S. federal collaboration involving the Environmental Protection Agency[32] (EPA, including its ToxCast Programme), the National Center for Advancing Translational Sciences[33] (NCATS), the National Institute for Environmental Health Sciences[34] (NIEHS), and the Food and Drug Administration[35] (FDA). The goal of Tox21 is to quickly and efficiently test whether certain chemical compounds have the potential to disrupt processes in the human body that may lead to adverse health effects, using *in vitro* high throughput screening and computational methods.

Scientists from the SEURAT-1 and Tox21 consortia met in 2013 to facilitate a practical exchange of scientific information between the projects with the intention of stimulating cooperation between partner organisations on the issues of animal-free testing and assessment. The discussions were organised in five sessions, including an introductory session for describing the structure and the strategic aims of the two projects, and four technical sessions, namely chemical inventories, *in vitro* assays and test systems, computational approaches, and chemical safety assessment. In a recent paper (Sipes et al., 2013), it was concluded that by broadly surveying both the chemical landscape and biological target space, patterns of biochemical activity could be identified which were associated with known chemical-target interactions through similarity analyses. Results from this large inventory of chemical-biological interactions can inform read-across methods as well as linking potential targets to molecular initiating events in adverse outcome pathways for diverse toxicities.

ECHA is participating in the Scientific Expert Panel of the project and established an internal expert group to provide smooth cohesion between the cutting-edge science and current regulatory practices.

A key output of SEURAT-1 will be the case studies: one will be an ab initio prediction as a 'proof of concept' for SEURAT-1, to help illustrate knowledge gaps. The other case study, which is the focus of ECHA's input, is to be based on read-across, i.e. to illustrate how such 'new approach' data can be used to improve the quality of read-across arguments (e.g. to increase the 'confidence' in the case, to extend the scope of read-across or to expand categories). A potential use of improved read-across for repeated-dose toxicity is the 2018 registration deadline for substances >10 tonnes per year, i.e. more read-across cases and categories (or bigger categories) could be developed.

OECD

As ECHA noted in its first report, the Organisation for Economic Cooperation and Development (OECD) is the main organisation for developing and validating both conventional and alternative test methods. The adoption of valid test guidelines by the OECD gives them international recognition and a possibility for regulatory use. More information on their recent activities is available from the OECD website[36]. ECHA has nominated experts to participate in various OECD projects, e.g. the test guideline development programme.

Due to ECHA's strategic aim to obtain high quality information for safe manufacture and use of substances through registration, the Agency intends to investigate how the OECD QSAR Toolbox, which aims to predict properties of substances, can contribute. In particular, preparing to meet the third registration deadline, a number of improvements in the Toolbox are planned in ECHA's multi-annual work programme that intend to facilitate the use of the software for estimating properties and filling data gaps for low tonnage industrial substances.

The grouping approach was adopted by the OECD in 1992 and the OECD Cooperative Chemicals Assessment Programme (CoCAP) was initiated.

31 http://epa.gov/ncct/Tox21/

32 http://www.epa.gov/

33 http://www.ncats.nih.gov/

34 https://www.niehs.nih.gov/

35 http://www.fda.gov/

36 http://www.oecd.org/env/ehs/

The programme aimed to minimise the duplication of work between the OECD member countries by involving the chemicals industry and non-governmental organisations in the chemical assessment process.

At the end of 2013, about 920 substances were assessed as per the OECD assessment. This is about a tenth of the number of substances that have been registered already under REACH at the time of this report. Of all the chemicals assessed in the CoCAP, over half were members of category assessments. Targeted assessments were also applied by the OECD. CoCAP has been revised to take into account the developments of several chemicals regulatory regimes in member countries and generally tighter resources. The focus of the programme is expected to shift towards more specialised hazard assessment activities after 2014.

Regulatory efforts aimed at harmonising the use of non-test methods are continuously ongoing. The OECD Guidance on grouping has been updated in April 2014[37]. It has been modified with the intent of improving readability and to give more guidance on e.g. the analogue and category approach, on quantitative and qualitative read-across, read-across justifications, on using bioprofiling activity in grouping chemicals. Chapter 6 of the updated OECD document would provide more guidance on the specific types of category approaches (e.g. chemicals of variable composition, metals, and manufactured nanomaterials). In this context, it is important to note that the OECD Guidance does not and will not replace the REACH Guidance on (Q)SARs and grouping of chemicals (Chapter R.6 of REACH Guidance on information requirements and chemical safety assessment[38]) but rather complements it.

An important step for the international community was adopting the concept that read-across is endpoint-specific. It is not that certain hypotheses and confirmed mechanisms cannot be used for several endpoints, but it is required for hypotheses and supporting evidence to address the given endpoint specifically due to the potential endpoint specific nature of the mechanism. As a consequence, every substance should be assessed for membership in a defined category for every endpoint, for which data gap filling by read-across is used. It follows that local and systemic effects must be considered separately. Potential deviations from the group due to kinetic and metabolic factors (Patlewicz et al., 2013)[39] should be considered and the resulting uncertainties covered by the approach.

There is a trend to describe and explain the fate and effects of chemicals in terms of pathway-based approaches. Different terms are being used to capture variants of this general framework, including source-to-outcome pathway, toxicity pathway (TP), mode of action (MoA) and adverse outcome pathway (AOP). While these terms are not yet harmonised, they are all based on the assumption that a toxicant, after reaching and interacting with a biological target, initiates a cascade of events which may lead to an adverse outcome at the organism or population level. The general premise of the AOP approach is that a limited set of key measurable events are sufficient for describing biological pathways and predicting adverse outcomes at multiple levels of biological organisation (cell, tissue/organ, organism, population). For practical purposes in chemical hazard and risk assessment, this means that a detailed molecular understanding of all possible molecular interactions and effects is not necessary, and that ultimately it may be sufficient for decision making to predict the adverse outcome at organism and population level from early ("upstream") key events.

Possible applications of the MOA/AOP approach include supporting read-across arguments in the analogue and category approaches, developing mechanistically based (Q)SAR models, developing mechanistically based *in vitro* tests, and building IATA.

37 http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en

38 http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

39 Patlewicz et al. (2013), Use of category approaches, read-across and (Q)SAR: general considerations. Regul Toxicol Pharmacol. 2013 Oct;67(1):1-12.

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU

ECHA-14-A-07-EN - ED-04-14-483-EN-N - ISBN: 978-92-9244-593-5 - DOI: 10.2823/22471