ECHA
EUROPEAN CHEMICALS AGENCY

The use of alternatives to testing on animals for the REACH Regulation

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

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FOREWORD BY THE EXECUTIVE DIRECTOR

Dear reader,

This is the third time that we report to the European Commission on how companies use alternatives to animal testing under the REACH Regulation.

Testing chemicals on animals should be the last resort – when there is no other scientifically reliable way to assess the potential effects of chemicals on humans or the environment.

This report confirms the findings of our earlier reports: data sharing works well and registrants make extensive use of different alternatives to animal testing, such as read-across, weight of evidence, computer modelling or *in vitro* and *in chemico* methods.

This is a positive trend. However, registrants bear the burden of proving the reliability of these alternative methods. We are concerned that, in many cases, the quality of information on alternatives in the submitted dossiers is not robust enough to replace animal tests and therefore we urge registrants to update their dossiers accordingly before evaluation.

We want to encourage registrants to use alternative methods in the best possible way. Over the last years, we have invested in giving advice and tools to industry to promote alternative methods and approaches so that the data would comply with the legal requirements.

In preparation for the final 2018 REACH registration deadline, we have published updated guidance, practical guides, case studies and webinars dealing with the topic. To help registrants improve the use of read-across – the most commonly used alternative method as identified in this report – we have published the read-across assessment framework. It shows how our experts assess read-across when evaluating registration dossiers.

Another development area are the new approach methodologies, where we held a dedicated workshop and continue giving input to scientific projects. We will follow and support the scientific development of methods that could limit or replace the need for new studies on animals in the long term. A priority are those methods which can increase confidence in read-across and improve its predictability.

To ensure that testing on animals is only done as a last resort, we also started in 2015 to ask all registrants submitting a proposal for testing chemicals on vertebrate animals to provide their considerations on alternative methods to us. This information is available for review by third parties on our public consultations web page before decision-making.

Looking back at 10 years of REACH implementation, I want to thank companies for the progress made so far. Human health, a safe environment and innovation are the main goals of REACH. Developing better alternatives to animal testing is an important issue for a humane and safe society. We will use this report's findings to promote the proper use of alternative methods and to support their further scientific development.

I hope that you will find the report of interest to you.

Geert Dancet,

Executive Director

EXECUTIVE SUMMARY

This is the third report on the use of alternatives to testing in animals by ECHA. The first two reports were published in 2011 and 2014[1].

The report is submitted to the European Commission to fulfil ECHA's obligation under Article 117(3) of the REACH Regulation. The analysis of the data contained in the registration dossiers provides up-to-date information on the use of alternative methods and testing strategies by registrants. The report also suggests opportunities to develop and use alternative test methods.

MAIN FINDINGS

Overall, the instruments provided by REACH to avoid unnecessary animal testing seem to work well. The main contributing factor is the registrants' obligation to share data and register jointly. This ensures that for each substance the test data are collected, generated and brought together in one joint registration dossier, instead of every potential registrant doing it individually.

Registrants make an extensive use of existing information and the various adaptation possibilities instead of conducting new studies or proposing new high tier vertebrate animal tests. In general, for the 6 290 analysed substances for the endpoints concerning vertebrate animals:

- 89 % contain at least one endpoint in the dossiers where an adaptation or other argument was provided instead of a study result;
- 63 % contain at least one read-across adaptation;
- 43 % contain at least one weight-of-evidence argument; and
- 34 % contain at least one QSAR prediction.

Based on the relative amount of experimental data available and adaptations used by registrants, three groups of endpoints can be identified: low tier endpoints, high tier human health endpoints and high tier environmental endpoints. Experimental data are available for 66 %, 40 % and 9 % of substances, on average across endpoints within the three groups, respectively.

For **low tier endpoints** (acute rodent toxicity, skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation and short-term toxicity to fish), the main source of information is experimental studies, with a high percentage of them carried out before REACH.

Less experimental data is available for **high tier human health endpoints** (repeated dose toxicity (all routes, all durations), genetic toxicity *in vivo,* developmental toxicity, toxicity to reproduction and carcinogenicity) compared to low tier endpoints. Read-across is the most used alternative approach, followed by weight of evidence.

For **high tier environmental endpoints** (bioaccumulation, long-term fish toxicity and long-term toxicity to birds), adaptations are much more common than experimental data and much less experimental data is available compared to the low tier endpoint short-term fish toxicity. Data waiving is used most frequently, followed by QSARs and read-across.

1 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

Overall, 11 % of REACH information requirements analysed in this report have been covered by new experimental studies performed on vertebrate animals.

Registrants already use existing alternative methods and approaches for **skin corrosion/irritation and serious eye damage/eye irritation** (over 56 % of the new studies performed for these endpoints were *in vitro* studies). In some cases, the information requirement was fulfilled by using *in vitro* test data either alone or together with other information, mainly existing *in vivo* studies with the registered substance or an applied read-across approach (about 20 %). However, registrants largely make use of existing *in vivo* studies only (>50 %), and to a lesser degree new *in vivo* studies only (up to about 4 %) to fulfil the requirements. ECHA has provided tools and practical guides on how to properly use newly developed methods and to build good quality QSAR predictions.

For **skin sensitisation**, registrants use experimental data, read-across strategies as well as non-animal test batteries, which avoid unnecessary animal testing. Non-animal test methods and the associated testing strategy for this endpoint have only recently been implemented in REACH. For the 2018 deadline, this testing strategy is the default approach.

Due to limitations in the above approaches (e.g. applicability domain of the method, or adequacy for classification and labelling purposes), some *in vivo* testing may still be necessary. However, some *in vivo* tests may be available for other reasons, for example, if registrants under REACH obtain access to such studies as they were conducted to fulfil regulatory requirements outside the EU. Where ECHA suspects that registrants have not complied with their legal obligations to use alternative methods, the Agency will, refer the case to Member State authorities to consider any enforcement action.

TESTING PROPOSALS AND CONSIDERATIONS FOR ALTERNATIVES

If registrants cannot provide the information required for high tier endpoints with existing experimental data, alternative methods or by omitting the study on justified grounds, they need to submit a testing proposal to ECHA and await its decision on the proposal. Since September 2015, registrants must also provide their considerations for alternative methods to justify the need for testing proposals on vertebrate animals.

So far, the vast majority of examined testing proposals were considered necessary by ECHA and the Member States and resulted in an adopted decision authorising the testing.

Feedback from investigations conducted by Member States suggests that registrants who have provided high tier studies did not violate obligations requiring enforcement actions to submit testing proposals.[2]

NEW ANIMAL TESTS SINCE START OF REACH

With the increasing number of new registration dossiers submitted to the Agency, the absolute number of new experimental studies has also increased for all endpoints. Nevertheless, alternative options for addressing information requirements have been used extensively by registrants.

This is particularly true for high tier endpoints, where the numbers for new experimental studies or testing proposals are not as high as could be expected from the number of submitted registration dossiers.

2 https://echa.europa.eu/chemicals-in-our-life/animal-testing-under-reach

QUALITY DEFICIENCIES IN THE USE OF ALTERNATIVE METHODS

ECHA's evaluation experience shows that many adaptations had quality deficiencies[3]. These include, especially with respect to commonly used read-across adaptations:

- poor documentation,
- insufficient substance identification,
- significant deficiencies in the quality of the source studies,
- lack of or low quality of supporting data,
- lack of qualitative and quantitative data to support predictions based on toxicokinetics, and
- shortcomings in the toxicological hypothesis.

The deficiencies related to the supporting evidence are particularly relevant for high tier human health and high tier environmental endpoints. To increase the robustness and regulatory acceptance of those adaptations for high tier human health endpoints, additional data is needed, particularly related to toxicological mechanisms and absorption, distribution, metabolism and excretion (ADME) properties.

New approach methodologies (e.g. high throughput *in vitro* screening) have a potential, to further substantiate the hypotheses of read-across approaches. As these approaches often use starting points which are directly relevant for humans (e.g. human liver cells), more relevant data can be obtained. One of the great challenges is currently to compensate the complexity of a higher organism (metabolism, toxicokinetics, etc.).

WORK TO PROMOTE ALTERNATIVE METHODS

ECHA will continue its efforts to promote alternative methods.

For low tier information requirements, there is a range of appropriate alternative *in vitro* methods already available. In addition, the amount of available experimental data and the generally less complex toxicology increase the possibility to successfully apply alternatives like read-across and QSARs. For these low tier endpoints, ECHA's focus is on promoting the available possibilities, especially in light of the 2018 deadline.

For high tier endpoints, the focus will be on making the shortcomings that are observed more explicit. Publication of the Read-Across Assessment Framework[4] allows registrants to improve their read-across predictions for these endpoints. The framework has been recently updated to cover environmental endpoints, and a separate report on considerations of multi-constituent substances and UVCB aspects was published in March 2017[5].

ECHA supports the development of the OECD QSAR Toolbox[6]. It is a software that can be used to support read-across. The Toolbox will be further developed and improved before the third REACH registration deadline in 2018, and beyond.

The development of new approach methodologies will bring high throughput assessment methods, which can support current alternative approaches, and might potentially provide more human relevant information. A

3 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports

4 https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

5 https://echa.europa.eu/-/how-to-consider-a-read-across-approach-for-multi-constituent-and-uvcb-substances

6 http://www.qsartoolbox.org/

challenge is to bring them into regulatory use. Where appropriate, ECHA will contribute to their development, for example, by giving regulatory input to projects and activities (e.g. EU Tox Risk).

ECHA will also continue to contribute to developing and promoting alternative methods in an international context, most specifically through the OECD.

PREFACE

This is the third report intended to meet ECHA's legal obligation under Article 117(3) of the REACH Regulation providing 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 two reports were published in June 2011 and in June 2014[7].

The primary source of information for these reports are registration dossiers submitted by manufacturers and importers to ECHA in the registration process. The results of ECHA's dossier evaluations (compliance checks and examinations of testing proposals) are another source of information but relate only to a fraction of the dossiers submitted. Such findings are also reported by ECHA in its annual evaluation progress reports, which, under Article 54 of the REACH Regulation, are published in February each year[8].

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. It is beyond the purpose of this report to analyse the reasons for low quality of used adaptations.

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.

7 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

8 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

LIST OF ABBREVIATIONS

3 Rs	Reduction, refinement, replacement of animal testing
ADME	Absorption, distribution, metabolism and excretion
AOP	Adverse outcome pathway
APCRA	Accelerating the pace of chemical risk assessment
CLP	Classification, Labelling and Packaging Regulation (EC) No 1272/2008
Commission	European Commission
JRC	Joint Research Centre
DPRA	Direct peptide reactivity assay
ECHA	European Chemicals Agency
EOGRTS	Extended one-generation reproductive toxicity study
ESR	Endpoint study record
EU	European Union
EU-ToxRisk	An integrated European flagship programme driving mechanism-based toxicity testing and risk assessment for the 21st Century
FET	Fish embryo acute toxicity
GLP	Good laboratory practice
h-CLAT	Human cell line activation test
HPLC	High pressure liquid chromatography
IATA	Integrated approach to testing and assessment
IR&CSA	Information requirements and chemical safety assessment
ITS	Integrated testing strategy
IUCLID	International uniform chemical information database
NAM	New approach methodologies
NES	New experimental studies (2009 and after for the purpose of this report)

NONS	Non-phase in substances, which have previously been notified under Directive 67/548/EEC and are documented in the European List of Notified Chemical Substances (ELINCS)
OES	Old experimental studies (before 2009 for the purpose of this report)
OECD	Organisation for Economic Cooperation and Development
OHT	OECD harmonised template
OSOR	One substance, one registration
PPORD	Product and process-oriented research and development
QSAR	Quantitative structure-activity relationship
RAAF	Read-Across Assessment Framework
REACH	Registration, evaluation, authorisation and restriction of chemicals
SIEF	Substance information exchange forum
SIP	Substance identification profile
TG	Test guideline
TMR	Test Methods Regulation (EC) No 440/2008
UES	Unique experimental study
UVCB	Substance of unknown or variable composition, complex reaction products or biological materials

LIST OF THE TERMS (GLOSSARY)

Adaptation	Adaptation of the standard information requirement means the use of non-animal methods for addressing or omitting information requirements.
Adverse outcome pathway	The sequence of events from the chemical structure of a target chemical or group of similar chemicals through the molecular initiating event to an *in vivo* outcome of interest.
Alternative approach	Encompasses use of alternative methods, integrated testing strategies (ITSs) or integrated approaches to testing and assessment (IATAs) to fulfil the standard information requirements specified in REACH.
Alternative (test) method	In the context of REACH, this mainly relates to use of *in vitro* methods, QSAR grouping and read-across (Article 13(1)): "Information on intrinsic that the conditions set out in Annex XI of the REACH Regulation 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)." An alternative test method can also be an *in vivo* test, but which uses fewer animals and/or causes less suffering.
Category	Group of substances with physico-chemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity.
Data	Data in this report means information submitted on the (eco)toxicological properties of chemical substances. The quality of data might be assessed depending on its source (e.g. if experimental or non-animal data). Good quality experimental data are usually derived from studies performed according to the principles of good laboratory practice (GLP) and are given Klimisch scores 1 or 2[9].
Data waiving	In this document, data waiving means omitting the standard information required for an endpoint either by means of the general REACH Annex XI adaptations (testing is not technically possible as defined in REACH Annex XI(2)) or based on considerations of exposure (REACH Annex XI(3)), or by specific Column 2 adaptations of REACH Annexes VII–X.
Defined approach	An approach to testing and assessment that consists of fixed data interpretation procedures used to interpret data generated with a defined set of information sources, that can either be used on its own, or together with other information sources within an integrated approach to testing and assessment (IATA).

9 https://echa.europa.eu/documents/10162/13628/evaluation_report_2016_en.pdf/f43e244f-7c90-75bd-e1b2-3771bcb1f8e8

Disregarded studies	Studies submitted by the registrant, but not used for the hazard assessment.
Endpoint	Observable or measurable inherent property/data point of a chemical substance. It may refer to a physicochemical 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). For the purposes of this report, low tier endpoints are considered to be those outlined in Annexes VII and VIII to REACH. High tier endpoints are considered to be those required in Annexes IX and X to REACH. In this report, two exceptions are made, which are the 28-day repeated dose toxicity and screening studies for reproductive developmental toxicity (Annex VIII requirements of the REACH Regulation), which are also counted as high tier endpoints.
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.
Experimental study	Experimental investigation set up to obtain information on a substance's intrinsic properties or adverse effects. It can cover *in chemico*, *in vitro* and *in vivo* testing.
Fingerprint	Technique, based on specific fields of a IUCLID dossier and used for computational analyses in this report to identify unique studies, which are reported more than once in the registration database.
Flag to omit the study	IUCLID flags to omit the studies 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 possible or necessary based on low exposure considerations.
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.
Integrated approach to testing and assessment (IATA)	An approach based on multiple information sources used to identify and characterise hazards and/or assess the safety of chemicals. An IATA integrates and weighs all relevant existing evidence and guides the targeted generation of new data, where required, to inform regulatory decision-making regarding potential hazard and/or risk. Within an IATA, data from various information sources (i.e. physicochemical properties, *in silico* models, grouping and read-across approaches, *in vitro* methods, *in vivo* tests and human data) are evaluated and integrated to draw conclusions on the hazard and/or risk of chemicals.
Integrated testing strategy (ITS)	An integrated and systematic approach to guide testing so that the sequence is not necessarily prescribed ahead of time but is tailored to the chemical-specific situation in such a way that the information gained in a testing sequence is maximised.

In silico	Information derived from or produced by using computer software or simulation, e.g. QSARs.
***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.
IUCLID flag	Option used in IUCLID to indicate a submitted data type (e.g. experimental data) or their use for regulatory purposes (e.g. confidentiality).
Lead registrant	One registrant acting with the agreement of the other assenting registrants who will submit the joint dossier.
Non phase-in substance	A substance which is not a phase-in substance within the meaning of Article 3(20) of the REACH Regulation. Non phase-in substances do not benefit from the transitional regime provided for phase-in substances under REACH and therefore have to be registered before manufacture or import starts.
Performance standards	Standards that provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar to a validated test method. This includes: essential test method components; a minimum list of reference chemicals used to demonstrate the acceptable performance of the validated test method; and comparable levels of accuracy and reliability that the proposed test method should demonstrate when evaluated using the minimum list of reference chemicals based on what was obtained for the validated test method.
Phase-in substance	A substance which meets at least one of the following criteria: (a) it is listed in the European Inventory of Existing Commercial Chemical Substances (EINECS); (b) it was manufactured in the Community, or in the countries acceding to the European Union on 1 January 1995 or on 1 May 2004, but not placed on the market by the manufacturer or importer, at least once in the 15 years before the entry into force of the REACH Regulation, provided the manufacturer or importer has documentary evidence of this; (c) it was placed on the market in the Community, or in the countries acceding to the European Union on 1 January 1995 or on 1 May 2004, before the entry into force of REACH Regulation by the manufacturer or importer and was considered as having been notified in accordance with the first indent of Article 8(1) of Directive 67/548/EEC but does not meet the definition of a polymer as set out in the REACH Regulation, provided the manufacturer or importer has documentary evidence of this.

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.
QSARs and SARs	QSARs are theoretical models that can be used to predict the physicochemical, biological (e.g. (eco)toxicological) and environmental fate properties of compounds in a quantitative or qualitative manner 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 QSAR is a mathematical model relating 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.
REACH-IT	Central IT system providing support for REACH processes.
Test (or assay)	Experimental system set up to obtain information on the intrinsic properties or adverse effects of a chemical substance.
Unique experimental study	A study, which for all fields included in the fingerprint appears unique, i.e. it contains non-repeating information compared to other studies.
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

1.1 BACKGROUND

The REACH Regulation[10] primary objective is to ensure that human health and the environment receive a high level of protection. This aim is also balanced with promoting alternative methods for assessing substance hazards, and the need to enhance the competitiveness and innovation of industry.

REACH prescribes the minimum information requirements for physicochemical, toxicological and ecotoxicological properties of a substance based on the tonnage of the chemical substances manufactured or imported.

Where more information on the intrinsic properties of substances is needed, tests have to be conducted according to the test methods laid down in a Commission Regulation[11] or in accordance with other international test methods that the Commission or ECHA recognise as being appropriate.

Information on intrinsic properties may also be generated in other ways than by tests, as long as the conditions set out in Annex XI to REACH are met, hereafter "REACH Annex XI". To address general requirements for generating information on intrinsic properties of substances, testing on vertebrate animals must only be undertaken as a last resort.

A key new feature introduced in REACH was to give greater responsibility to registrants for ensuring safety. REACH is based on the principle that manufacturers, importers and downstream users are responsible for ensuring and showing that they manufacture, place on the market, or use substances that do not adversely affect human health or the environment. Therefore, industry is responsible for generating the necessary information to properly identify and manage the hazards and risks. REACH stipulates the minimum information requirements that must be fulfilled in Annexes VII to X to REACH, hereafter "REACH Annexes VII to X".

ECHA[12] was established for managing the implementation of the legislation and, in some cases, carrying out the technical, scientific, and administrative aspects of REACH. It also has to ensure consistency at EU level with respect to these activities. ECHA helps companies comply with the legislation, advances the safe use of chemicals, provides information on chemicals and addresses chemicals of concern.

1.2 SCOPE

Under Article 117(3) of the REACH Regulation, "the Agency, in accordance with the objective of promoting non-animal testing 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 focus in this report was analysing how the registrants used adaptations (alternative methods and data waiving).

10 https://echa.europa.eu/regulations/reach/legislation

11 http://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32008R0440

12 https://echa.europa.eu/about-us

The first report was published in June 2011. The methodology developed for that report was also applied to the second report (published on 2 June 2014)[13], with some methodological improvements and with new types of information included. This third report uses further improved methods to allow for a deeper and more refined analysis on how registrants addressed their information requirements under REACH.

Companies report information on the substances they manufacture or import in a registration dossier submitted to the Agency. The level of information to be submitted depends on the substance tonnage and/or its hazardous properties. The registration dossier must be in IUCLID format. For this report, ECHA analysed IUCLID registration dossiers for all four tonnage bands (i.e. 1-10 tonnes per annum (tpa), 10-100 tpa, 100-1 000 tpa, and 1 000 tpa and above) corresponding to each respective REACH information requirement in REACH Annexes VII-X.

The majority of the substances in the tonnage bands 1 000 tpa and above (REACH Annex X) and 100-1000 tpa (REACH Annex IX) were already registered by 2010, and 2013, respectively.

However, for the analysis of information as required in REACH Annex VII (1-10 tpa) and REACH Annex VIII (10-100 tpa), the vast majority of registrations is still to come since the registration deadline for these substances is May 2018. Moreover, comparisons with previous reports results should be undertaken with caution because registration dossiers at the 1-10 tpa and 10-100 tpa tonnage bands have not been included in prior analyses.

The total data pool analysed for this report contains substances registered between 1 June 2008 and the cut-off date of 31 March 2016. It covers registrations submitted from the two previous registration deadlines, as well as registrations already submitted to cover the information requirements of REACH Annexes VII and VIII. It, therefore, provides a suitable pool to analyse the use of alternative methods on the one hand and the most up to date information available on the other.

The analyses are based on dossiers submitted by lead registrants, because their dossiers usually contain the full hazard data package for the joint submission. Only a few member dossiers contained hazard information due to the possibility to opt-out from the general joint submission obligation, and these dossiers were also included in the analysis.

In line with the previous two reports, certain submissions were again excluded from the scope. These included registrations submitted for:

i) substances only used as intermediates under strictly controlled conditions;

ii) substances notified for use in product and process-orientated research and development (so-called "PPORDs"); and

iii) 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 in this series. Furthermore, only endpoints related to vertebrate animal testing have been analysed.

13 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

1.3 OUTLINE

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

Section 1 explains the scope of the current report in terms of period and analysed data.

Section 2 outlines REACH instruments to avoid unnecessary animal testing.

Section 3 contains detailed analysis of the various options chosen by registrants to fulfil standard information requirements.

- Section 3.2.1 analyses the main options used.
- Section 3.2.2 shows how often adaptations are used per endpoint. The detailed results explaining whether adaptations have been used as a principal option, or together with other evidence, are given in Appendix 4.
- Section 3.2.3 summarises the *in vitro* test results for the endpoints skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation. More details are given per endpoint in Appendices 5, 6 and 7, respectively.
- Section 3.2.4 provides results on the analysis of data availability, i.e. the count of new experimental studies reported under the REACH Regulation. A comparison between old and new experimental studies for the submissions related to REACH Annexes VII-X is shown in Appendix 8. The table also specifies which test guidelines were used.
- Section 3.2.5 provides results on the analysis of testing proposals. Testing proposals were analysed at different phases of the regulatory process (submission, evaluation and follow-up).
- Appendix 1 describes the methodology applied for analysing the registration dossiers.
- Appendix 2 gives a detailed breakdown of the information extracted from the registration dossiers per endpoint and per option selected for filling the information requirements.
- Appendix 3 illustrates what type of information registrants submitted by year during the study period.

Section 4 details the findings of this report, incorporating references from other ECHA reports and including elements of forward thinking and willingness to incorporate scientific achievements in ECHA's operational practice. Scientific initiatives in support of registrants are described. Needs to further promote alternatives are also discussed.

2. LEGAL INSTRUMENTS TO AVOID UNNECESSARY TESTING

The REACH Regulation provides different ways to avoid unnecessary testing and ensure that new vertebrate animal testing is only undertaken as a last resort. The main instruments are data sharing, adapting information requirements and testing proposals.

2.1 DATA SHARING AND JOINT SUBMISSION

All registrants of the same substance have to share data related to vertebrate animals. If they cannot reach an agreement, they can submit a dispute to ECHA, which may give them access to data, if appropriate. ECHA also provides data when it has been submitted more than 12 years previously. In this case, the data can be re-used freely by others for registration.

Data sharing applies to old experimental studies as well as new studies conducted either spontaneously by registrants to fulfil an information requirement, in preparing their registration dossier or updating it, or after receiving a request from ECHA following an evaluation decision.

There are two possible routes for data sharing: pre-registration and establishment of substance information exchange forums (SIEFs) for existing (phase-in) substances and inquiry to ECHA for all other substances. Pre-registration ends on 31 May 2017 for phase-in substances under certain conditions[14]. After this date, the inquiry route remains the only way to get in contact with other registrants of the same substance.

New contacts between companies for sharing data have continued since the previous report. For phase-in substances, on average 14 000 pre-registrations were received each year in the period 2014-2016 for getting access to the SIEFs. For new substances or phase-in substances that are new on the EU market, an average of about 1 500 registrants benefitted from the inquiry service each year. Furthermore, the majority of companies have respected the 'one substance, one registration' (OSOR) principle and submitted their registration jointly. Dossiers submitted outside the joint submission concerned only 2 % of substances registered for all uses[15].

However, some issues still need to be addressed to ensure that data sharing and joint submission are respected by all parties. In substance identification, improvements were needed to provide clarity on the substance registered jointly and their link to the tested material.

In anticipation of the 2018 registration deadline, the Commission issued an Implementing Regulation[16] in 2016 to clarify the data-sharing principles and the requirement that ECHA must ensure that all registrants of the same substance are part of the same joint submission, even where a registrant separately submits some information (opt-out). This prompted the need to revise the Guidance on data-sharing[17]. ECHA also improved its IT system, REACH-IT, to prevent submissions outside of existing joint submissions. This ensures that co-registrants discuss sharing of all relevant data for the substance and avoid duplicating animal tests.

14 Phase-in substances below 100 tonnes per annum, within six months after exceeding the one tonne per annum threshold.

15 https://echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf/4c958d7a-3158-447b-9e81-d8bae9a7e7f9

16 Commission Implementing Regulation (EU) 2016/9 of 5 January 2016 on joint submission of data and data-sharing in accordance with Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

17 https://echa.europa.eu/documents/10162/13631/guidance_on_data_sharing_en.pdf/545e4463-9e67-43f0-852f-35e70a8ead60

It is also worth noting that in recital 15 of the Implementing Regulation the sharing of animal studies that are conducted on a substance, which is structurally similar to the substance being registered (for grouping or read-across) is encouraged in order to promote the development and use of alternative methods for assessing hazards of substances and avoiding unnecessary animal testing.

Regarding substance identification, an improved reporting format was implemented in IUCLID 6 for documenting the identity and the detailed composition of the substance registered as well as the tested substance for each endpoint study record. This is essential as different compositions may have different fate and hazard profiles and may require additional studies to cover those properties. This information is also critical for adequately predicting the properties and effects of the substance from those of another one when registrants fill data gaps by using alternative methods, e.g. read-across or QSARs.

2.2 ADAPTATION POSSIBILITIES OF REACH

REACH provides different options for deviating from the standard testing regime and using alternative methods, provided they are duly justified and scientifically sound. These options are listed as possible adaptations in REACH Annex XI(1) and include:

1) use of existing data, including historical human data;
2) use of a weight-of-evidence approach;
3) information generated using quantitative structure-activity relationships (QSARs);
4) *in vitro* test methods; and
5) grouping of substances and read-across.

Adaptations can be used either individually or combined in a weight-of-evidence approach (e.g. use of QSAR and information from read-across in combination with literature evidence and/or some properties indicating the possible fate of a substance). In all cases, the data used must be adequate, reliable and relevant for the particular endpoints, and must follow the criteria set out in Annex XI.

It is also possible to omit (i.e. waive) the standard information required for an endpoint by other means than the options listed above. REACH Annex XI provides data-waiving possibilities when testing is not technically possible (REACH Annex XI(2)) or based on exposure considerations (e.g. no significant exposure can be shown) (REACH Annex XI(3)).

In addition, for some endpoints, Column 2 of REACH Annexes VII-X gives specific rules for other adaptation or data-waiving possibilities (e.g. based on considerations of other hazardous properties).

For the analyses conducted for this report, omitting studies as a result of REACH Annexes VII-X, Column 2 adaptations is not distinguished from omitting studies according to REACH Annex XI adaptations. Thus, both options were assigned a label "flags to omit studies". For this report, the terms "omit study" and "data waiving" were used as synonyms.

2.3 TESTING PROPOSALS AND THIRD PARTY CONSULTATIONS

For the purposes of registration under REACH, registrants must not undertake any new studies involving vertebrate animals (REACH Annex IX or X) before submitting a testing proposal to ECHA and receiving ECHA's decision. When they submit their proposal, the registrants must show in their IUCLID dossier that they have considered alternatives[18].

ECHA organises third party consultations for all testing proposals involving vertebrate animals, for the endpoints specified in REACH Annexes IX and X. The aim is to ensure that there is no scientifically valid existing data that could address the hazard endpoint covered by the testing proposal. Such information, if it can be used in filling the data gap, may mean that the proposed testing is no longer required and is sent to the registrant together with the draft decision for their consideration. ECHA, in consultation with the Member States, adopts the decision based on the registrant's proposal, the information submitted by third parties and any readily available information identified by ECHA.

Many comments received from third parties are about potential strategies that the registrant could use e.g. information supporting weight of evidence, reference to open literature and, more rarely, potentially relevant studies. However, the registrant may face challenges to make use of this information. One difficulty is to get reliable and adequate documentation so that the information can be used for classification and risk assessment and has adequate and reliable coverage of the key parameters addressed in the corresponding test method. Another challenge is to get access to study reports identified by third parties and compensate the data owner.

Despite more than 800 comments received in 2014-2016, the impact of third party consultations has remained relatively limited for the reasons outlined above. In addition, the number of consultations receiving third party comments has noticeably declined in 2015-2016[19]. Nevertheless, in annual evaluation progress reports, ECHA has provided examples of third party comments, which triggered the registrants to change their testing strategies[20,21]

18 https://echa.europa.eu/view-article/-/journal_content/title/considerations-for-alternative-methods-need-to-be-included-in-your-testing-proposal

19 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

20 https://echa.europa.eu/documents/10162/13628/evaluation_report_2012_en.pdf/fa360388-4c23-4816-90be-09812061e12f

21 https://echa.europa.eu/documents/10162/13628/evaluation_report_2013_en.pdf/e080ba36-64a6-4dcf-8eca-f9352ddf5e3b

3. ANALYSIS OF ENDPOINT DATA SUBMITTED IN THE REGISTRATION DOSSIERS

This section outlines the analysis performed on registration dossiers falling under the scope of this report. The methods used for the previous report in this series (published on 2 June 2014)[22] have been further improved to allow for a deeper and more refined analysis of how registrants addressed their information requirements under REACH.

3.1 METHODS

In total, 6 911 dossiers (see Table 1) containing hazard information corresponding to 6 290 substances were analysed in detail for this report.

Table 1: Registration dossiers with hazard information within the scope of this report for all tonnage bands (6 911 dossiers in total).

	> 1 000 tpa	100 - 1 000 tpa	10 - 100 tpa	1 -10 tpa
Non phase-in	125	198	379	821
Phase-in	2 170	2 092	518	608
Totals	**2 295**	**2 290**	**897**	**1 429**

As explained in Section 1, the registration dossier must be submitted in IUCLID format, which enables robust study summaries to be prepared that describe how the information requirements were fulfilled by the registrants, either through experimental studies or alternative methods. For each inherent property of the substance (or endpoint), the information must be recorded in the endpoint study records (ESRs). There may be more than one ESR submitted per endpoint.

How standard information requirements and adaptation options are recorded in IUCLID is complex, and therefore, to present a comprehensive view of these data, more than one analysis of the data was performed (see Figure 1).

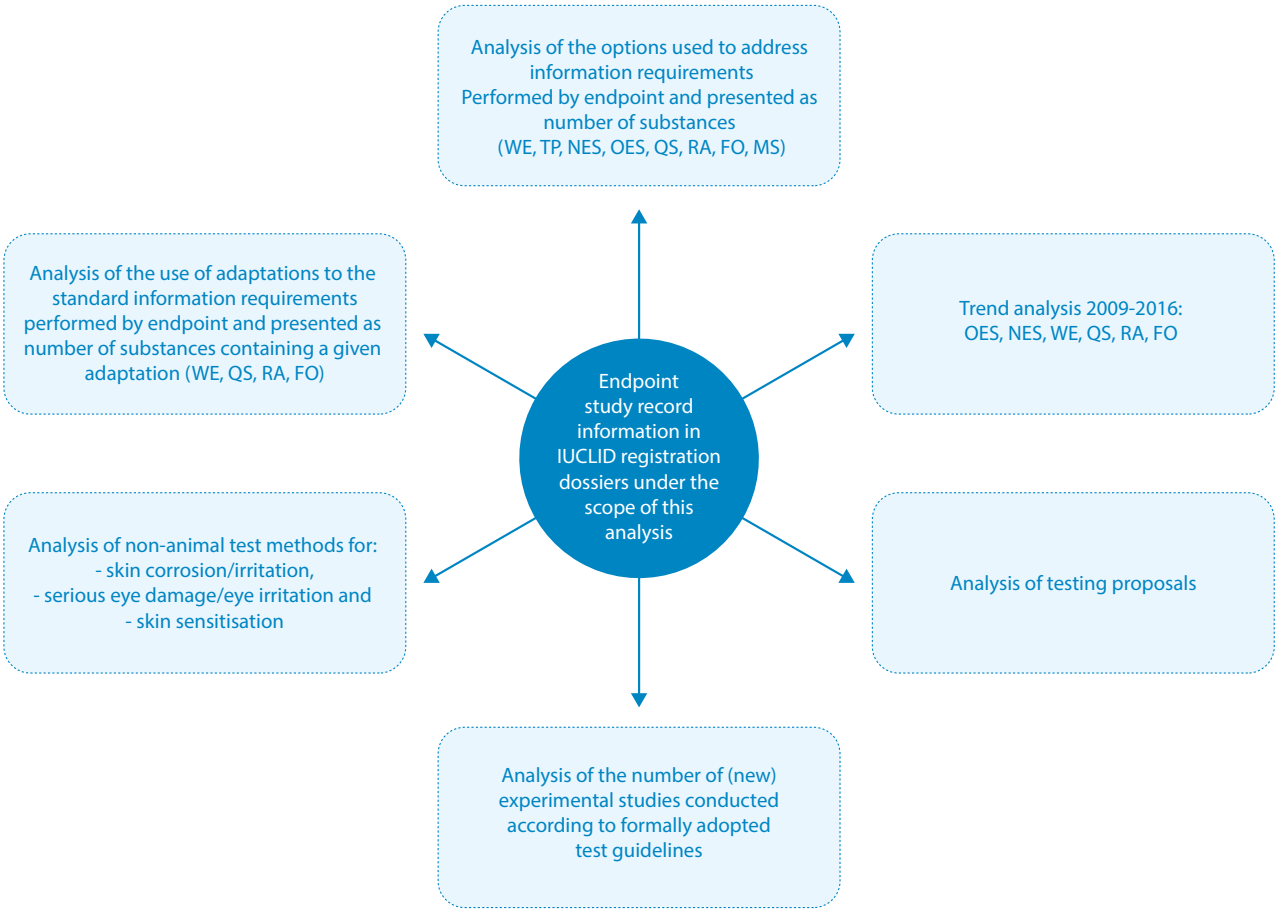
The analyses are based on IT algorithms and have largely not been manually verified. It is assumed that registrants have reported the information in the appropriate fields. This means that there is some level of uncertainty connected to the exact figures.

Analysis of the options used to address information requirements
This analysis gives an overview of the different options, which were used for each registered substance.

As dossiers may be regularly updated, the ESRs from registrants' latest submissions were used to provide the most up-to-date representation of the data in ECHA's central IUCLID database. The options analysed (in preferential order) were: weight of evidence (WE), testing proposals (TP), new experimental studies (NES), old experimental studies (OES), QSARs (QS), read-across (RA) and data waiving (FO). Details on which IUCLID fields were used to analyse these options are given in Appendix 1.

22 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

Figure 1: Overview of types of analysis conducted for the purpose of this report. (Legend: OES – old experimental studies (conducted before 2009); NES – new experimental studies; WE – weight of evidence; RA – read-across; QS – QSAR; TP – testing proposal; FO – flags to omit study; MS – miscellaneous)



If different options have been used for a specific endpoint, the following rules have been chosen to identify the main strategy that assumingly drove the registrant to address the information requirements:

- 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 a testing proposal included, this was taken as evidence that the endpoint was supposed to be filled by future testing.
- If there was one ESR entry referring to an experimental study, this was taken as evidence that the endpoint was filled with experimental data (with one exception: 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). If new experimental data (with a report date of 2009 or later) was submitted together with old experimental data (with a report date 2008 or before), the endpoint was considered to be filled by new experimental data.

- If there was no ESR entry referring to an experimental study but listing either a possibility to omit the information (data waiving) or to fill the information requirements using alternative approaches, it was counted as evidence that the endpoint was addressed by (Q)SARs, read-across, or data waiving.

The approach and rules described above were already used in the two previous reports in this series[23]. However, this report introduces a novelty: a distinction is made between new and old experimental data, which are considered as different strategies to address information gaps. New experimental data refers to studies with a report date of 2009 or later while old experimental data refers to studies with a report date of 2008 or earlier.

The result of this analysis is presented in Figure 2 in Section 3.2.1. It gives the relative proportions of options used by the registrants across all substances considered for this report: new experimental study, old experimental study, testing proposal, read-across, QSAR, weight of evidence and data waiving. Details on the method are given in Appendix 1, Section 1.1.

Analysis of the use of adaptations to the standard information requirements
ECHA analysed the use of adaptations to the standard information requirements as detailed in Column 2 of REACH Annexes VII-X and Annex XI (i.e. weight of evidence, read-across, QSAR and flags to omit the studies).

In contrast to the approach described above where a hierarchy across possible options was used, this analysis explores the use of adaptations in general, without distinguishing between its use as principal option or as supporting evidence. To highlight which adaptation was used most frequently for a given endpoint, the number of substances per adaptation was divided by the total number of substances with hazard information for this endpoint and sorted according to decreasing percentage; see Figure 3 in Section 3.2.2.

The total number of substances is different for each endpoint. It was used under the assumption that the number of substances for which information on the endpoint was given is approximately the number of substances for which there is also a standard information requirement. The difference may be related to additional data submitted by the registrant based on triggered information requirements or relevant and available existing data. Details on the method are given in Appendix 1, Section 1.2. A detailed breakdown of the results is given in Appendix 4 (Table 4.5).

Analysis of non-animal test methods for skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation
This part focused specifically on those endpoints for which regulatory accepted *in vitro* methods are now fully available, namely skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation.

Such test methods, if used within the limitations of their applicability, can be used to generate information to fulfil the REACH Annex VII information requirements either alone or in a testing and assessment strategy. Furthermore, depending on the methods used and the outcomes of the tests, the resulting information may allow for conclusions on classification and risk assessment based solely on these methods, without the need to perform an *in vivo* study.

The numbers of *in vitro* and *in vivo* studies for the above-mentioned endpoints were checked manually. Attention was paid to the general trends of use of *in vitro* methods, especially when registrants used them alone to fulfil their information requirements. Details on the method are given in Appendix 1, Section 1.3. The results of the analysis are given in Section 3.2.3 and in the Appendices 5-7.

23 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

Number of (new) experimental studies conducted according to formally adopted test guidelines
An analysis was undertaken on how many new and old experimental studies were submitted in accordance with internationally accepted test guidelines. The same endpoint study record may be used for more than one substance, e.g. if the study was conducted for a common constituent of different UVCBs. Therefore, a fingerprint methodology was applied with the aim of counting the same study only once.

The results are referred to as unique experimental studies (UESs). Details on the fingerprint methodology and on the method in general are given in Appendix 1, Section 1.4. Results on the availability of new experimental studies are given in Section 3.2.4, and details on old and new experimental studies are given in Appendix 8.

Testing proposals
An analysis of testing proposals was conducted. The outcome of testing proposals, which have been accepted by ECHA, will not be known until the deadline for submitting any required information has expired. In a number of cases, this is still several years away.

However, to provide a better insight on how these testing proposals are progressing, the analysis included several different stages in the life cycle of the proposals. These stages are data submission, dossier evaluation and follow-up to dossier evaluation.

- Data submission stage: this analysis aims to provide a measure of the original intentions of registrants who considered new experimental studies as necessary. Therefore, all ESRs containing testing proposals related to vertebrate tests which have been submitted until 31 March 2016 have been counted.

- Dossier evaluation stage: testing proposals can be removed, considered as inadmissible and therefore not further processed, put on hold, replaced by adaptations or appear under the scrutiny of the appeal procedure. Therefore, information concerning the examined proposals was collected from the evaluation units, see Table 3.

- Follow-up to dossier evaluation stage: it is not possible to provide an accurate analysis of the outcomes of the examination of testing proposals based on automated searches of registration dossiers and to associate them with the number of new experimental studies found in the automated search of registrations. To illustrate the outcome of the testing proposals, information from the follow-up examinations has been reported.

Details on the method are given in Appendix 1, Section 1.5. The results of the analysis are presented in Section 3.2.5.

Trend analysis
Finally, based on submitted ESRs from 2009-2016, an analysis was conducted to assess the trends of the following options: new and old experimental studies, weight of evidence, read-across, QSAR and data waiving. Only ESRs in registration dossiers submitted for the first time were included in the analysis, i.e. updates were not taken into account. To avoid bias introduced by the peaks around the deadlines, the number of ESRs were normalised by the total number of dossiers submitted per year and endpoint. Additionally, the cumulative number of ESRs submitted per year was added to the graphs. Details on the method are given in Appendix 1, Section 1.6. The result of the analysis is presented in Section 3.2.6.

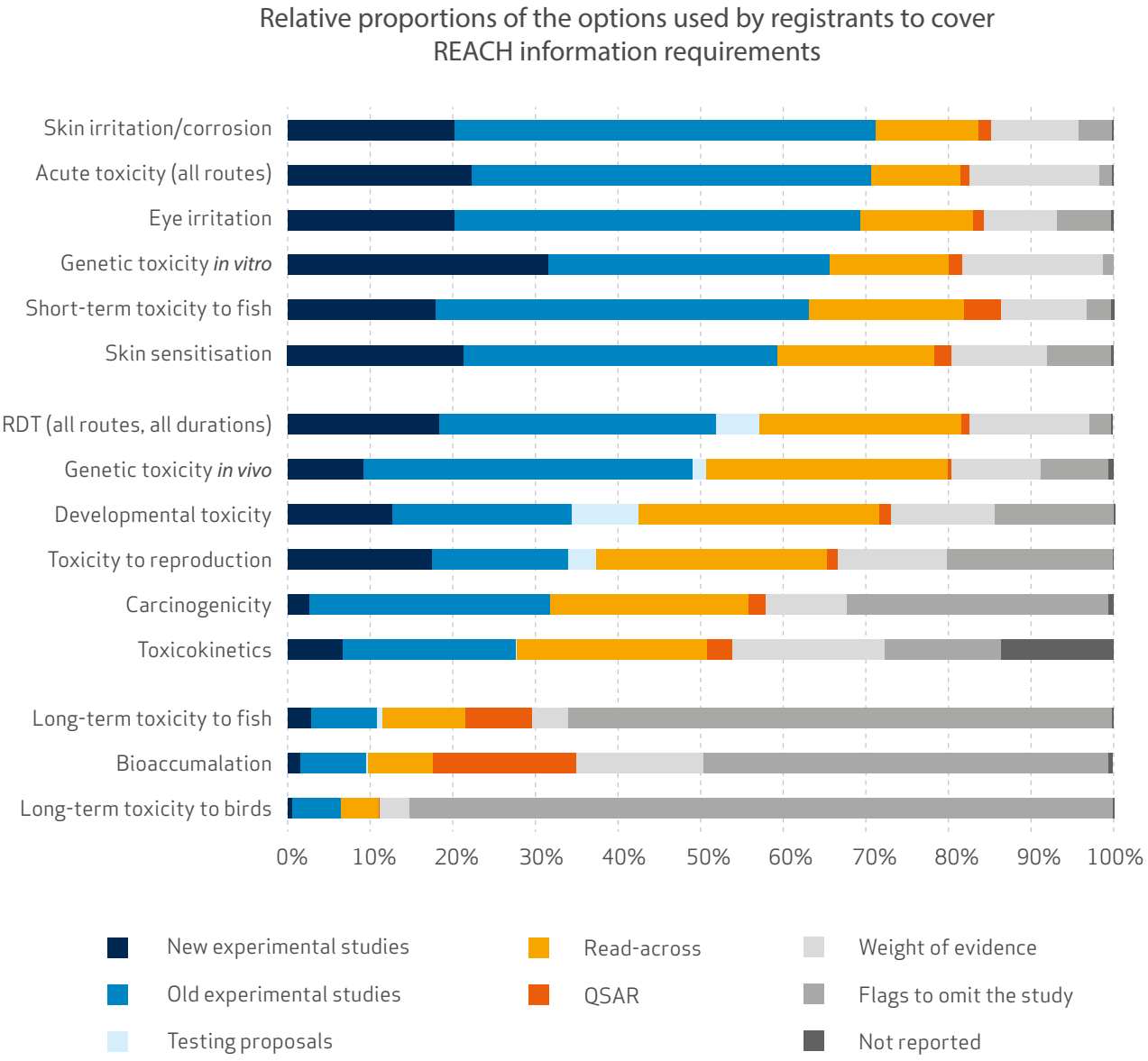
A detailed overview of the submitted ESRs presented per endpoint and type of option used to address the REACH information requirements is given in Table 2.1 in Appendix 2. A second table (Table 2.2 in Appendix 2) further distinguishes between phase-in and non phase-in status of the substance and which REACH annex relating to standard information requirements applied for the dossier.

3.2 RESULTS

3.2.1 Results on options used to address information requirements

This analysis gives an overview on the principal options (experimental studies, testing proposals and adaptations) used by the registrants to address their information requirements.

Figure 2: Relative proportions of the principal options to fulfil information requirements for human health and environmental endpoints for the substances. The total number of substances per endpoint for which at least one option has been submitted was taken as 100 %.



Overall, 11 % of REACH information requirements analysed in this report have been covered by new experimental studies performed on vertebrate animals. This is the average number of substances across all

analysed endpoints (for the endpoints skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation the number of substances with a new *in vivo* study is estimated based on the analysis given in Appendix 5-7).

Based on the relative amount of experimental data available, three groups of endpoints can be identified: low tier, high tier human health and high tier environmental endpoints.

For the purpose of this report, a distinction between low tier and high tier endpoints was made according to the following considerations. Endpoints outlined in REACH Annexes VII and VIII are considered as low-tier endpoints, while endpoints listed in REACH Annexes IX and X are considered as high tier endpoints.

However, 28-day repeated dose toxicity and screening studies for reproductive/developmental toxicity (REACH Annex VIII requirements) are also considered as high tier endpoints. This is due to the many similarities in biological parameters covered by these tests and similar challenges related to the application of alternative methods.

Results for high tier human health endpoints

High tier human health endpoints include repeated dose toxicity (RDT) (all routes, all durations), genetic toxicity in vivo, developmental toxicity, toxicity to reproduction and carcinogenicity. The availability of information on toxicokinetics, was included in that group for comparison only as the respective standard information requirement does not require the generation of new toxicokinetic data but relies on the assessment of available information, although this is often essential information to support read-across.

High tier human health endpoints are relatively data poor and adaptations were used frequently. Experimental studies for the analysed high tier human health endpoints were provided, on average, for 41 % of substances. Of those, old experimental studies were submitted for 27 % of substances, while there were new experimental studies for 13 % of substances.

The substances with new experimental studies in this group varied from 3 % for carcinogenicity to 18 % for repeated dose toxicity. For toxicity to reproduction, developmental toxicity and genetic toxicity *in vivo*, 17 %, 13 % and 9 % substances had new experimental studies, respectively.

In general, there were more old experimental studies compared to new experimental studies, varying between 17 % for toxicity to reproduction and 40 % for genetic toxicity *in vivo*. The only exception is toxicity to reproduction where the proportions of the new and old experimental studies were equal (17 %). The fraction of testing proposals compared to other principal options was generally low and varied between 2 % (genetic toxicity *in vivo*) and 8 % (developmental toxicity).

From all options to fill the data gaps, the testing proposals represent the smallest fraction. For this analysis, the most up-to-date dossier of a given substance was considered. The number of testing proposals counted is decreasing compared with previous reports. This is normal as once a test is performed and results are submitted, testing proposals are removed from the dossier, thus they do not appear in the dataset anymore. This has to be taken into account when comparing the fraction of testing proposals in this analysis with the total number of testing proposals presented in Section 3.2.5, where testing proposals were counted cumulatively up to 31 March 2016.

Adaptations to standard information requirements were widely applied for high tier human health endpoints. Read-across was the most frequently used option and, depending on the endpoint, varied between 24 % (for carcinogenicity and repeated dose toxicity) and 29 % (for developmental toxicity and genetic toxicity *in vivo*).

These results show that read-across was used more frequently for high tier human health endpoints than for other groups of endpoints. The next most frequently used adaptation is weight of evidence, which varied from 10 % for carcinogenicity to 15 % for repeated dose toxicity. Data waiving had larger variations across this group (from 3 % for repeated dose toxicity to 32 % for carcinogenicity). QSARs were used less frequently than any other adaptations for high tier human health endpoints, with most QSARs being used for carcinogenicity (2 %).

Results for high tier environmental endpoints
The group of the high tier environmental endpoints includes long-term toxicity to fish, bioaccumulation, and long-term toxicity to birds.

It is apparent from Figure 2 that experimental data were submitted by the registrants only for a small fraction of the substances. Experimental data account for less than 11 % for any of the high tier environmental endpoints, of which less than 3 % are new experimental studies. The fraction of testing proposals is below 1 %.

The most frequent option to address a standard information requirement was data waiving (49 % for bioaccumulation, 66 % for long-term toxicity to fish, and 85 % for long-term toxicity to birds). This is partially due to the tiered or conditional information requirements, i.e. the need to fulfil an information requirement on an endpoint depends on the properties of, or exposure to, the substance. Therefore, very frequently, standard information requirements for high tier environmental endpoints are waived. Reasons for data waiving were:

- conditions for triggering the information requirement on bird toxicity are not fulfilled,
- existence of specific rules for adapation from Column 2 of REACH Annexes VII-X (e.g. bioaccumulation test does not need to be performed for substances that have a low potential for bioaccumulation, for instance based on a low octanol/water partition coefficient, and/or a low potential to cross the biological membranes),
- use of ITS for long-term fish toxicity which also includes invertebrate species. For example, a number of conclusions for the environment can be derived from long-term toxicity to daphnids, which are not included in this analysis since daphnids are not vertebrate organisms.

The second most frequent option was QSAR (17 % for bioaccumulation, 8 % for long-term toxicity to fish, and 0.1 % for long-term toxicity to birds).

In general, read-across was used less frequently than for high tier human health endpoints (from 5 % for long-term toxicity to birds, to 10 % for long-term toxicity to fish). For long-term fish toxicity, read-across was the prefered option over QSAR. Weight of evidence was used most for bioaccumulation (15 %). For long-term toxicity to fish and birds, the frequency was 4 %.

Results for low tier endpoints
In the case of low tier endpoints covering human health and the environment (acute rodent toxicity, skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation and short-term toxicity to fish), the main source of information originates from new and old experimental data.

New experimental studies varied between 18 % for short-term fish toxicity and 22 % for acute toxicity (all routes). Old experimental studies, which were the principal option to fill the data gaps, varied from 38 % for skin sensitisation to 51 % for skin corrosion/irritation. Read-across on average was used in 15 % of substances where information was given for the endpoint. Weight of evidence was used between 9 % (eye

irritation) and 15 % (acute toxicity (all routes)). On average, 4 % of substances for low tier endpoints relied on data waiving, and 2 % on QSARs. The majority of QSARs were used for short-term toxicity to fish (5 %).

The main source of information originates from experimental *in vivo* studies (67 % on average), with a high percentage of them carried out before REACH (on average 44 %). For these endpoints, which are relatively data rich, there is a lower reliance on adaptations. Genetic toxicity *in vitro*, which is by definition an *in vitro* endpoint, was included in this group for comparison only. For these endpoints, read-across and weight of evidence were the predominant adaptations of standard information requirements. Short-term toxicity to fish is also assigned to this group (between genetic toxicity *in vitro* and skin sensitisation, based on the amount of experimental data available).

3.2.2 Results on the use of adaptations to the standard information requirements

The results for the legal adaptation possibilities of weight of evidence, read-across, QSAR and data waiving are presented in Figure 3. The figure shows the fraction of substances for which an adaptation was used related to the overall number of substances containing any data for this endpoint (total number of substances is therefore different for the different endpoints).

Overall, for endpoints related to vertebrate animal tests, data waiving is used most often, followed by the use of read-across, weight of evidence and QSAR.

While the fraction for data waiving and read-across varies considerably among the endpoints (5-90 % and 8-53 %, respectively), weight of evidence was used across all endpoints at a similar level (less than 16 %). QSAR was used frequently for bioaccumulation (for 31 % of substances), whereas the use of QSAR for other endpoints is relatively small (from 0 to 10 % of the substances).

Data waiving was used extensively for endpoints where multiple routes of administration are possible but are not required in the majority of cases. These endpoints include acute rodent toxicity (all routes) and repeated dose toxicity (all routes). For environmental endpoints, the high percentage of data waiving is partially due to the tiered or conditional information requirements as already explained in the previous section.

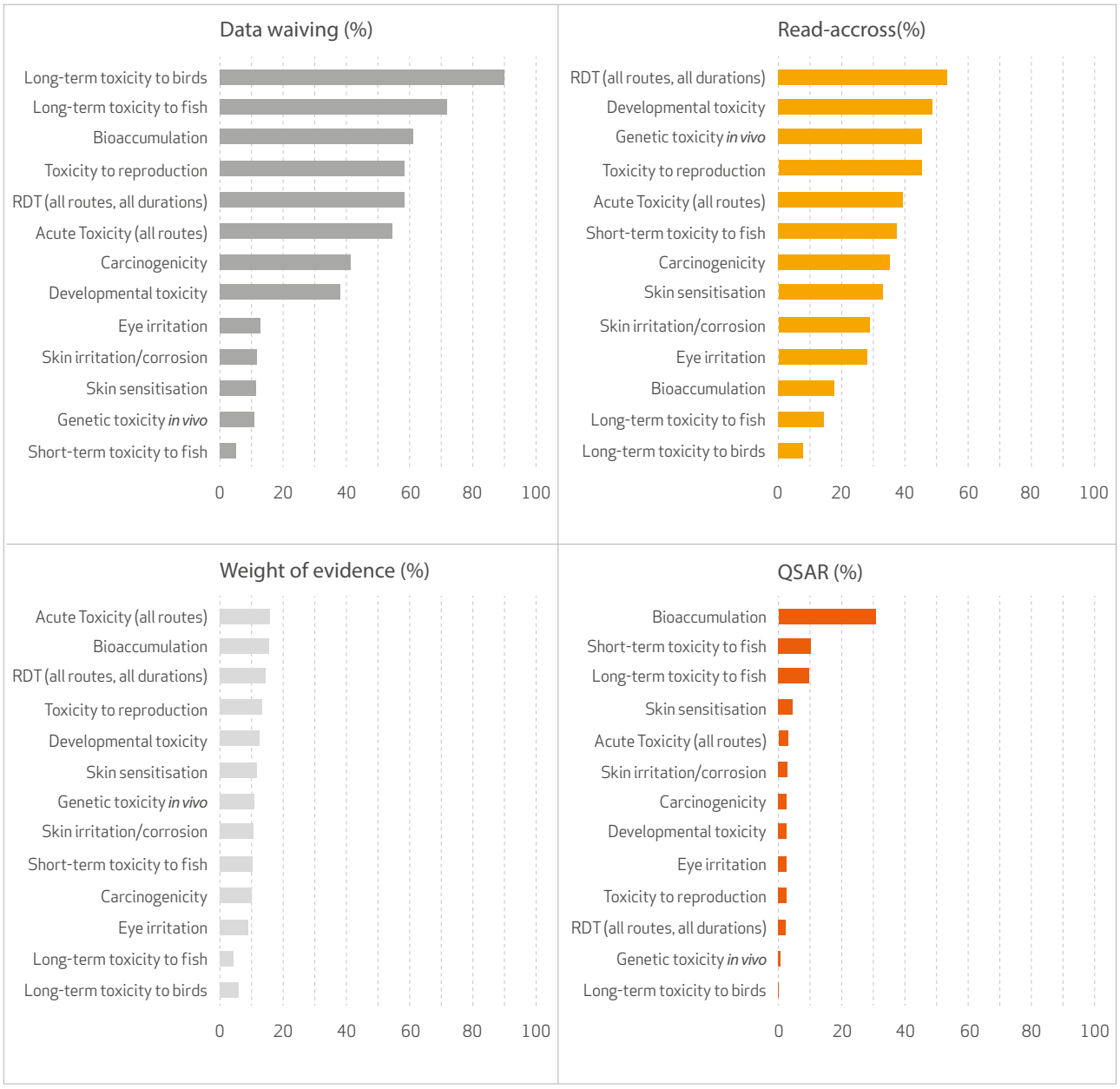
Toxicity to reproduction studies are those which are most expensive and require the use of many animals. This endpoint was frequently addressed by either data waiving or read-across, with a small amount of weight-of-evidence adaptations. Similar observations can be made for adaptations used for developmental toxicity.

Read-across was most frequently used for the high tier human health endpoints: repeated dose toxicity, developmental and reproductive toxicity, and genotoxicity *in vivo*. This observation is also made in the general analysis of options used by registrants (Section 3.2.1), but here this becomes more evident, as read-across is counted even if it was used as supporting evidence. Read-across is also considered a viable adaptation for complex health endpoints, presuming that a scientifically plausible hypothesis can support this and is used for deriving an adequate quantitative result.

For more than half of the substances which contained information on the endpoint repeated dose toxicity (all routes and all durations combined), read-across was used either to fill an information gap as the principal option, or together with other evidence. In general for all analysed endpoints, about 40 % of read-across endpoint study records (ESRs) were used as a key study, another 40 % as a supporting study, and in about 20 % of the cases, read-across was used in a weight-of-evidence approach (see Appendix 4 for further details).

Weight of evidence was used across all endpoints at a relatively low level. Table 4.3 in Appendix 4 gives details on how weight of evidence was used. It shows that weight of evidence most often contained read-across, but also old experimental studies when taking into account the total numbers for all analysed endpoints.

Figure 3: The fraction of substances for which an adaptation was used related to the overall number of substances with information for this endpoint. The endpoints are sorted in decreasing order of percentages and start with the endpoint where the adaptation was used most



QSARs were predominantly used for bioaccumulation and toxicity to fish. For almost a third of the substances for which the endpoint bioaccumulation had been addressed, QSAR was used to fill a data gap either on its own or together with other evidence. More detailed results are provided in Appendix 4 showing

the use of QSAR as a key study, supporting study or weight of evidence. They can be used to explore the difference between the use of an adaptation as the principal option (as presented in Section 3.2.1), and their overall use (as presented in Section 3.2.2).

Results from this analysis of the legal adaptation possibilities to the standard testing regime show that, in general, 89 % of the substances contain in the dossier at least one endpoint where an adaptation or other argument was provided instead of a study result, 63 % contain at least one read-across adaptation, 43 % contain at least one weight of evidence argument, and 34 % contain at least one QSAR prediction in the analysed endpoints concerning vertebrate animals. The numbers do not amount to 100 % because the information submitted for each endpoint in a substance could contain multiple adaptations and therefore substances can be counted several times.

3.2.3 Results from the analysis of non-animal test methods for skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation

Detailed analysis, including manual verification, for skin corrosion/irritation and serious eye damage/eye irritation endpoints (for all registrations within the scope of this analysis, calculated as a percentage of the number of substances with information on the endpoint) showed that registrants mainly used existing data (about >50 %) or solely read-across approaches (about 15 %). Almost 20 % of the substances contained *in vitro* studies, either as the sole source of information or provided in combination with other sources of information. New *in vivo* tests alone were found for 3.2 % (skin corrosion/irritation) or 4 % (serious eye damage/eye irritation) of the substances analysed.

The total number of *in vitro* studies submitted for skin corrosion/irritation and serious eye damage/eye irritation endpoints has increased from 1 940 (in the previous report) to 2 918 by end of March 2016. This increase mainly relates to the inclusion of registrations submitted for tonnages below 100 tpa (REACH Annex VII and VIII dossiers) to the pool of analysed substances, whereas in the previous report only registrations submitted for tonnages above 100 tpa (REACH Annex IX and X dossiers) were analysed.

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 based on manually verified results, the approaches taken by registrants appear to follow the currently described practices by OECD and ECHA for using IATA: if *in vitro* test results are inconclusive, *in vivo* tests may be necessary.

Concerning the skin sensitisation endpoint, the use of *in vitro/in chemico* methods has slightly increased when compared to the previous report (102 vs 54 ESRs submitted in 2014) probably due to the recent adoption of new *in vitro/in chemico* test guidelines. Due to the recent REACH information requirement revision, the use of *in vitro/in chemico* test methods is expected to increase in the future.

Registrants have been reminded that, if they have existing *in vivo* data available, they do not need to conduct new *in vitro/in chemico* studies. However, in such a case they have to add a data-waiving record addressing the endpoint and to provide a justification in a IUCLID dossier.

Detailed analyses of the skin corrosion/irritation, serious eye damage/eye irritation and skin sensitisation are provided in Appendices 5, 6 and 7, respectively.

3.2.4 Results on number of new experimental studies conducted according to formally adopted test guidelines

As mentioned earlier, new experimental studies with vertebrates must only be conducted if there is no adequate existing information and alternative methods cannot be applied reliably. Therefore, to indicate the extent to which this principle is being pursued by registrants, the generation of new experimental information used to address an information requirement under REACH was further investigated.

The number of substances for which new experimental studies were submitted, cannot be directly translated to the number of new studies conducted. Many studies have been used for several substances, e.g. if the study was done with common constituents of various UVCBs. Therefore, a specific analysis to identify "unique" new experimental studies (with a report date 2009 or later) was carried out for this report. Experimental studies not conducted according to internationally accepted test guidelines were excluded from the analysis.

The total number of new *in vivo* studies for endpoints related to vertebrate animals is 9 287 (versus 4 887 in 2014). The total number of old experimental studies conducted according to internationally accepted test guidelines is 2.5 times higher than the number of new experimental studies (Appendix 8) for the endpoints analysed. The total number of new *in vitro* studies is 5 795 (vs 3 052 in 2014). Over 56 % of the new experimental studies performed for the endpoints skin corrosion/irritation and serious eye damage/eye irritation are existing alternative methods and approaches.

Among the *in vivo* endpoints, there is a considerable increase of new experimental studies since the previous analysis for the endpoints pre-natal developmental toxicity (359 vs 82 counted for the 2014 report) and repeated dose toxicity (90-day, all routes) (268 vs 79 counted for the 2014 report). These are also the endpoints for which experimental data have been most often requested in ECHA's evaluation decisions.

Due to the change in the REACH annexes, the extended one-generation reproductive study (EOGRTS) is now a standard information requirement in REACH Annex X, and can be proposed for substances with information requirements according to REACH Annex IX, if there is concern from screening or other studies.

ECHA has issued draft decisions requesting information to fulfil the information requirements for the reproductive toxicity endpoint. Due to the lack of consensus within the Member State Committee on the design of the studies to be requested, 216 draft decisions were passed to the Commission for decision making. The Commission is currently processing the draft evaluation decisions that ECHA had referred to it.

A common approach was established involving ECHA and the Member States. Cases will be grouped in the Commission decisions but addressed individually to the registrants. Therefore, the majority of results related to the EOGRTS testing can be expected only after 2020.

The use of new *in vitro* methods was analysed in detail, with results given in Section 3.2.3 and in Appendices 5, 6 and 7. Therefore, the number of new *in vitro* studies is not further commented in this section.

Table 2: New experimental studies with report date of 2009 or later. Detailed information on study types are shown in Appendix 8.

Endpoint Name	Type/ Species usually tested	Annex X	Annex IX	Annex VIII	Annex VII	Total
Skin corrosion/irritation[a]	*in vitro*[e]	305	559	153	401	1 418
Serious eye damage/eye irritation[a]	*in vitro*[e]	214	410	130	334	1 088
Skin sensitisation[a]	*in vitro*[e]	18	39	17	28	102
Genetic toxicity	*in vitro*	841	1 194	588	564	3 187
Total number of "new" experimental studies *in vitro*						**5 795**
Bioaccumulation	fish	23	19	12	5	59
Short-term toxicity to fish	fish	254	467	219	120	1 060
Long-term toxicity to fish	fish	75	39	19	11	144
Long-term toxicity to birds	bird	4	0	7	0	11
Acute toxicity (oral)	rat or mouse	189	335	143	345	1 012
Acute toxicity (inhalation)	rat or mouse	126	110	44	32	312
Acute toxicity (dermal)	rat or mouse	160	366	141	76	743
Skin corrosion/irritation[b]	rabbit	155	271	126	189	741
Serious eye damage/eye irritation[b]	rabbit	309	474	192	242	1 217
Skin sensitisation	mouse and guinea pig	369	555	177	416	1 517
Genetic toxicity	rat or mouse	83	108	64	42	297
Carcinogenicity	rat or mouse	13	0	2	0	15
Repeated dose toxicity 28-day (oral)	rat or mouse	64	168	66	49	347
Repeated dose toxicity 28-day (inhalation)	rat or mouse	44	21	9	5	79
Repeated dose toxicity 28-day (dermal)	rat or mouse	5	6	1	4	16
Repeated dose toxicity 90-day (oral)	rat, mouse or rabbit	105	73	25	8	211
Repeated dose toxicity 90-day (inhalation)	rat or mouse	32	13	6	1	52
Repeated dose toxicity 90-day (dermal)	rat or mouse	2	1	0	2	5
Repeated dose toxicity chronic	rat or mouse	2	0	3	0	5

Endpoint Name	Type/ Species usually tested	Annex X	Annex IX	Annex VIII	Annex VII	Total
Combined chronic repeated dose toxicity with carcinogenicity	rat or mouse	8	6	4	0	18
Repeated dose toxicity other[c]	rat or mouse	8	18	10	6	42
Combined repeated dose toxicity 28-day with screening and screening study	rat or mouse	184	444	290	34	952
Pre-natal developmental toxicity	rat or mouse	184	123	38	14	359
Toxicity to reproduction: one-generation	rat or mouse	7	8	4	0	19
Toxicity to reproduction: two-generations	rat or mouse	15	8	0	0	23
Toxicity to reproduction other [d]	rat or mouse	15	11	5	0	31
Total number of "new" experimental studies *in vivo*						**9 287**
Miscellaneous experimental studies (toxicokinetics, developmental neurotoxicity, other)	not specified	35	45	23	3	106
Total number of "new" experimental studies						**15 188**

[a] These endpoints were manually verified.
[b] There might be other guidelines than *in vivo* skin or eye irritation reported under these sections e.g. acute dermal toxicity or skin sensitisation, which may have formed a basis for waiving.
[c] Other guidelines (not included in OHT 2016) reported under 7.5.x (oral, inhalation, dermal), including few neurotoxicity studies in rodents.
[d] Guidelines reported under reproduction and fertility effects (7.8.2) not included in OHT 2016 edition.
[e] It is noted that for this specific table, the term "*in vitro*" studies covers any non-animal test (i.e. *in chemico*, *in vitro* and *ex vivo* studies).

3.2.5 Testing proposals submitted to and evaluated by ECHA

As explained in Section 2.3, it is through the process of examining a testing proposal that ECHA decides in consultation with the Member States whether any proposed vertebrate testing to fulfil the information requirements of REACH Annexes IX and X is necessary.

The generation of information resulting from adopted evaluation decisions on testing proposals can take several years, due to the timeframe needed for completing some vertebrate animal studies and sequential testing strategies. At this point in time, it is therefore not possible to report the outcome of all testing proposals already submitted. However, detailed information on the status of testing proposal examinations is already provided in the annual evaluation progress reports[24] and ECHA's second report on the operation of REACH and CLP[25], published in June 2016.

24 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

25 https://echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf/4c958d7a-3158-447b-9e81-d8bae9a7e7f9

It is only when an expert individually examines the registration that any testing proposal can be verified. For the purposes of this review, there are some useful insights from the aforementioned reports which give some context to the outcomes of testing proposals and the use of alternative methods. Especially so, as such information cannot be obtained using IT tools to examine the registration database.

As a starting point, the computational search of the registration database showed that, up to 31 March 2016, registrants had originally submitted 1 827 testing proposals for endpoints concerning vertebrate animal testing. A description of the algorithm is given in Section 3.1. This number is a maximum estimate for testing proposals, as some testing proposals may have been withdrawn before they were processed.

Apart from some exceptional cases with ambiguous substance identity issues, ECHA has examined within the legal timeframe all testing proposals submitted for the first two registration deadlines for phase-in substances. The analysis of 1 488 testing proposal examinations conducted up to 31 December 2016 (an update of the information provided in the 2016 Report on the Operation of REACH and CLP) showed that 25 % were terminated before the adoption of the decision: 212 cases were terminated before a draft decision was issued and 165 cases after sending of the draft decision.

As discussed below, the available information so far shows that the majority of testing proposals examined by ECHA were considered necessary and resulted in an adopted decision authorising the testing. However, there is a significant number in which the original intention to propose testing does not result in new experimental study data on the registered substance.

Registrants may remove their testing proposals for a number of justifiable reasons, for example, if they become aware of new data, which can allow a new adaptation possibility, or if a test conducted for other regulatory purposes becomes available (e.g. by letter of access).

Moreover, in some cases, and for some endpoints, the outcome of a testing proposal might be conditional on a sequential testing strategy (e.g. for genetic toxicity *in vivo*, the outcome of *in vitro* tests may alter the need for the *in vivo* test). Finally, registrants may also have business reasons such as a change in the volume of production, or cease of manufacture leading to termination of the testing proposal examination.

As of 31 December 2016, there were 953 information requests stemming from adopted decisions for endpoints concerning vertebrate animal tests (see Table 3 below). It is not possible to directly correlate these requests with the number of animal tests that may result. Such requests may address sequential testing strategies involving the prior conduct of invertebrate tests or may accept the use of data from tests conducted with another substance (e.g. read-across) as plausible. The most frequent requests are for information for pre-natal developmental toxicity studies and repeated dose toxicity 90-day studies.

In respect of testing proposal evaluations resulting in information requests for the endpoint toxicity to reproduction, 183 draft decisions were referred to the Commission due to the lack of unanimous agreement to the proposed tests within the Member State Committee.

Table 3: Total number of requests for tests in adopted decisions with testing proposals (2009-2016)[a]

Endpoint (concerning vertebrate animals only)	Total number of requests in adopted decisions with testing proposals (2009-2016)[b]
Bioaccumulation	18
Long-term toxicity to fish	48
Repeated dose toxicity (all routes)	359
Mutagenicity/genotoxicity *in vivo*	55
Pre-natal developmental toxicity	467
Toxicity to reproduction[c]	6
Total	**953**

[a] Figures from ECHA's second Report on the Operation of REACH and CLP[26] and updated to December 2016.
[b] Includes requests for information from other substances and sequential testing strategies.
[c] The Commission is currently processing the draft evaluation decisions that ECHA had referred to it.

The time to generate new information is set in the adopted decisions. If several tests are requested, the deadline allows for any necessary sequential testing. In some cases, sequential testing may allow an adaptation to be developed removing the need to complete the full set of tests. This timeline can be between one and four years for typical combinations of high tier tests, depending on their type and the number of tests.

As of 31 December 2016, 629 requests from ECHA for information on the registered substance or an analogous substance following examination of vertebrate animal testing proposals were awaiting expiry of deadlines set in the adopted decisions. In addition, there are a number of yet to be adopted decisions on testing proposals for vertebrate animal tests. Therefore, it is anticipated that some new tests may only become available by 2020 or later, assuming no unexpected delays.

Once the deadline set in the decision expires, ECHA examines whether the request for information is met, in accordance with REACH Article 42. The available information shows that the majority of updated dossiers were compliant with the decisions[27].

The testing proposals have also included several large categories of substances. Based on the read-across approach proposed by the registrant and concluded by ECHA as plausible, testing of only some of the substances was considered necessary. In a number of these cases, the outcomes are still to be assessed.

Companies who propose tests involving vertebrate animals need to show that they have fully considered alternative methods before concluding that a new animal test is necessary. Since 21 June 2016, a dossier with a proposal to test on vertebrate animals needs to have documented such considerations of alternatives for each proposed vertebrate study to pass the completeness check.

This practice follows a European Ombudsman decision about ECHA's role in evaluating testing proposals.

26 https://echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf/4c958d7a-3158-447b-9e81-d8bae9a7e7f9

27 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

3.2.6 Tests performed without a testing proposal

Based on the data analyses conducted for the first and the second report in this series[28], ECHA noted that some new high tier studies were conducted without submitting a testing proposal or without an evaluation decision from ECHA.

As outlined in the second report, ECHA analysed reasons why some registrants submitted 295 studies on vertebrate animals without having submitted a testing proposal and without awaiting the ECHA decision. This analysis showed that registrants may have valid reasons why the studies were available to them and could be included in their registrations. These reasons, however, may not be apparent from the information provided in the dossiers. In those cases where registrants transparently provided their reasons, it became easier to judge whether a testing proposal should have been submitted or not.

Following the findings of the second report, a more detailed analysis of these 295 cases was performed. The outcome explaining the reasons these 295 studies were available was published on ECHA's website in July 2015[29]. For example, the tests were conducted for other regulatory purposes (82) or conducted by a legal entity other than the lead registrant (57), among others. In 15 cases, it appeared that registrants may have misunderstood obligations to submit testing proposals for new *in vivo* mutagenicity tests to fill information requirements. ECHA subsequently made this obligation more explicit in its Guidance.

A number of cases of potential interest (121) were highlighted, as the reasons for the availability of the studies were either unclear or complex (86), the tests were conducted by other legal entities which may have obligations under REACH (31) or for reasons of "responsible care" (4) where the test was conducted, for example, to guarantee safe use of the chemical substance to downstream users.

When ECHA observes that a registrant has performed or is performing a high tier vertebrate test without having sought a prior agreement from ECHA, the Agency informs the relevant Member State authorities. This gives the authorities the opportunity to consider the need for any necessary investigations and enforcement actions, in accordance with REACH Articles 125 and 126.

ECHA invited the Member State authorities to provide feedback on their investigations of the cases above. Inspections by 7 of the 15 contacted Member States did not find any incompliance with the obligation to submit a testing proposal in 23 of 25 of the cases of potential interest. In 12 other cases investigated, no incompliance has been identified. An incompliance was confirmed in one case of potential interest and two others were not concluded at the time of reporting.[30]

This suggests that, in general, registrants are not avoiding obligations to submit testing proposals. ECHA will inform the Member State authorities of any new cases that are found during evaluation.

3.2.7 Examples of testing proposals and applied read-across approach

There can be a potential saving in the number of high tier tests using the category and read-across approach, when it is applied in an appropriate manner, offering the same level of protection for human health and the environment.

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28 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

29 https://echa.europa.eu/documents/10162/13628/analysis_higher_tier_without_tp_results_en.pdf/055eb6fb-2fd7-49cc-877d-a19de53c3fc4

30 https://echa.europa.eu/documents/10162/13628/ms_investigations_testing_proposals_en.pdf/4d083086-006d-a8b4-0814-fd483fc22cb0

Taking into account substantial efforts by both ECHA and industry, it is noteworthy that, during the discussions on testing proposal examination, including during Member State Committee meetings, ECHA adopted decisions, which accommodated well defined testing strategies proposed by registrants for several larger groups of substances. This means that tests would be done only for some substances within the group, and the results would be read-across to the other substances in the group with data gaps. These were listed in the previous report in this series[31], and include 12 cobalt salts, 27 alkenes, 22 petroleum substances, and 35 hydrocarbon solvents.

The testing proposals for cobalt salts identified in the previous report were already concluded with adopted decisions sent to the registrants confirming their testing strategy regarding the 90-day repeated dose toxicity study (rat, oral), and pre-natal developmental toxicity (rats or rabbits, oral). The testing proposals concerning the two-generation reproductive toxicity study were separated from the decisions and referred to the European Commission in September 2013.

Regarding the petroleum substances mentioned above, the Member State Committee agreed with ECHA not to accept the registrant's category-based approach, but it could accept the testing plan as being plausible based on a one-to-one read-across. In all other cases (cobalt salts, alkenes, and hydrocarbon solvents), the Member State Committee agreed to the read-across approach proposed by the registrants and reflected in ECHA's draft decisions.

The uncertainties still present in the approach were recognised and the outcome of the testing strategy would only be assessed once the information would have been generated and examined. For bitumens and asphalt, it was agreed that a pre-natal developmental toxicity study would be performed with one of the analogues. The other tests requested concerned two-generation reproductive toxicity studies, which were referred to the Commission.

The outcomes of the testing strategies accommodated in the evaluation decisions for large groups for the above-mentioned substances will be subject to examination in the follow up procedure. Due to the extended timelines needed to generate the data, information on the results of many of these proposals have yet to be concluded.

There are some other read-across and grouping approaches used in testing proposals, where read-across was thought possible from the initial testing proposal evaluation. For example, read-across was accepted for acetalisation products between glucose and long chain alcohols. While tests with registered substances were agreed for endpoints that do not involve vertebrate testing (e.g. adsorption/desorption and earthworm reproduction test), a new 90-day rat oral repeated dose toxicity study was agreed to be conducted for only one of the two substances.

Read-across was also accepted in the case of some organic carboxylic acids in the form of their sodium salts for a 90-day inhalation rat repeated dose toxicity. This was based on the hypothesis that the toxicity of these chemicals is plausibly mediated by the organic ion, absorption of the organic part from various salts is equivalent (assuming that the cation does not have toxicity on its own), and this is supported by mechanism of action investigations and toxicity measurements in animal studies.

In all cases, however, if the testing strategy and resulting information do not confirm the read-across hypothesis relied upon by the registrants, this does not alter the obligation of the registrants to meet the standard information requirements. Should the read-across strategy be inadequate, it remains the registrants' responsibility 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

31 https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf

proposed approach does not satisfy the conditions set out in the REACH Annex XI, ECHA reserves the right to request the information necessary to fulfil the information requirements as mentioned above.

3.2.8 Findings from other evaluation processes

New tests can also be requested by ECHA as a result of compliance checks. As reported in ECHA's second Report on the Operation of REACH and CLP[32], approximately half of all compliance checks in 2009-2015 were concluded with a decision (722 of 1 536). Further, it was also reported that taking into account overlaps between substance and dossier evaluation processes at least 800-1 000 substances have been checked to a reasonable extent and missing key information has been requested for 19-24 % of substances registered at 100 tpa or above. Not all of the requests concern high tier endpoints – more details are available in ECHA's evaluation progress reports.

In relation to compliance checks performed on the most important endpoints for assessing an impact on human health and environment (eight super endpoints[33]), the most frequent requests for human health endpoints were: genotoxicity (*in vitro* and/or *in vivo* tests) – 128, pre-natal developmental toxicity – 113, repeated dose toxicity – 73, reproductive toxicity – 17 and carcinogenicity – 1.

For environmental endpoints, long-term aquatic toxicity was the most frequent – 36, followed by biodegradation – 12, and bioaccumulation – 3. This finding is supporting evidence that, in many cases, the registrants did not consider a testing proposal necessary but neither succeeded to justify and document their adaptations adequately.

3.2.9 Trend analysis

A trend of the options chosen by registrants when they submitted a registration dossier for the first time for a given substance was analysed for 2009-2016.

Across all endpoints analysed, a general decrease of the use of existing experimental information can be identified, from almost 60 % in 2009 to less than 30 % in 2016. This is counterbalanced mainly by a slight increase for weight of evidence (4 % in 2009 to 22 % in 2016) and new experimental studies (5 % in 2009 and 13 % in 2016). Read-across seemed to have a small peak in 2013 with 32 %, with the fraction of read-across being lower in 2009 (15 %) and 2016 (21 %).

The fractions for QSAR, data waiving and testing proposals were low throughout the years compared to the other options used and did not show a specific trend over time in their use. Graphs showing the trend for each option are presented in Figure 3.1 in Appendix 3.

The use of the above-mentioned options for the single endpoints follow more or less the same pattern, and therefore separate graphs for the endpoints were not included in this report.

32 https://echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf/4c958d7a-3158-447b-9e81-d8bae9a7e7f9

33 https://echa.europa.eu/documents/10162/13608/echa_cch_strategy_en.pdf/607b157b-a35d-4d1c-8e62-ce8668324b1a

4. THE USE AND PROMOTION OF ALTERNATIVES

One of the main reasons for developing REACH was that a large number of substances were already in use on the European market for many years, and there was inadequate information on their intrinsic properties and the risks that their use may pose.

The REACH Regulation balances the need for generating new information on intrinsic properties of chemical substances by using animal tests, with the provisions to avoid unnecessary animal testing. Consequently, the legislation emphasises the principle that testing on vertebrate animals must be undertaken only as a last resort after exhausting all other options for adapting the testing requirements, such as use of existing data, *in vitro* methods, read-across from similar substances, or application of QSARs.

The availability of suitable alternatives to animal testing and the proper use of these alternatives are, however, essential to achieve the primary objective of REACH, namely, ensure a high level of protection of human health and the environment from the hazardous effects of chemicals. The extent to which adaptations to animal testing are used and aspects of their quality are discussed in this chapter.

4.1 USE OF ALTERNATIVES TO ANIMAL TESTING

Given that not only the use, but rather the proper use of alternatives to testing on animals is essential to achieve a key objective of REACH, it is also important to reflect upon on the quality of adaptations. Therefore, recently published reports which address quality are included in the discussion below.

In ECHA's second report on the operation of REACH and CLP[34], published in June 2016, it is stated that while companies make extensive use of alternatives to testing on animals, this was often poorly justified and documented and can ultimately result in the need to do tests after all. ECHA's evaluation experience over recent years has shown that the use of read-across was often found to be problematic[35].

In general, the reasons for insufficient quality of adaptations, especially with respect to commonly used read-across adaptations, were: poor documentation, insufficient substance identification, deficiencies in the quality of the source studies, lack of or low quality of supporting data, lack of qualitative and quantitative data to support predictions based on toxicokinetics, and shortcomings in the toxicological hypothesis. The deficiencies related to the supporting evidence are particularly relevant for high tier human health and high tier environmental endpoints.

To bring the adaptations to the level required by REACH for high tier human health endpoints, additional data, especially that related to toxicological mechanisms and absorption, distribution, metabolism and excretion (ADME) properties, are needed in many cases.

ECHA supports registrants to improve justifications for adaptations by providing an extensive description of deficiences in its evaluation decisions. Furthermore, ECHA proactively promotes and supports registrants by providing numerous supporting materials which explain critical elements especially for the use of read-across and QSAR. More details on ECHA's promoting activities are given in Section 4.2.

QSAR was mostly used for the environmental endpoints bioaccumulation and fish toxicity (short and long-term), and rarely for human health endpoints compared to other options. Easy to use computational

34 https://echa.europa.eu/documents/10162/13634/operation_reach_clp_2016_en.pdf/4c958d7a-3158-447b-9e81-d8bae9a7e7f9

35 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

software tools are available for predicting the bioaccumulation potential and fish toxicity. This may be one of the reasons for the relatively high use of QSARs for these endpoints. It is noted that for some types of substances it is however challenging, or even not possible to make reliable bioaccumulation predictions based on log Kow relationships, such as for inorganic substances, surface active substances, and ionisable substances.[36] For fish toxicity, especially for long-term toxicity to fish, limitations can come from specific chemical structures and a lack of sufficient experimental data for substances similar to the one to be predicted.

For the majority of low tier endpoints there is an obligation for some, and a significant opportunity for others, to use alternative methods. Using QSAR predictions to address short-term toxicity to fish may become more reliable due to the accessibility of large databases with experimental data and increasing knowledge on the relationship between hydrophobicity and toxicity.

QSAR predictions for skin sensitisation are related to the possibility to predict to some extent the skin permeability and key events in the sensitisation pathway such as protein binding, which determines the reactivity pattern of substances. The grouping of substances by their protein binding potential allows application of local models, derived for specific target chemicals (e.g. as in the OECD QSAR Toolbox).

However, QSAR adaptations cannot accurately predict the outcome of tests where the quantitative result is based on multiple adverse biological events, which is the case for high tier endpoints. For example, QSARs for fulfilling a standard information requirement for repeated dose toxicity generally do not exist or are of unknown/low quality, due to the complexity of the endpoint.

For some endpoints (e.g. acute oral toxicity, skin irritation/corrosion, serious eye damage/eye irritation, skin sensitisation), there are recently approved testing strategies available which use non-vertebrate animal tests and can replace or reduce the number of tests on vertebrate animals. In addition, ECHA has provided a plethora of practical tools and guidance on how to properly use adaptations and to build good quality predictions[37],[38].

ECHA has brought possible issues of compliance with the obligation to use alternative methods to the attention of the Member States. To explore possible options for addressing these incompliances, ECHA has conducted two compliance checks requesting registrants to justify why alternative methods (e.g. *in vitro* methods for skin corrosion/irritation and serious eye damage/eye irritation) were not used. ECHA will communicate the outcome of the follow-up to dossier evaluation decisions to the Commission and the Member States.

Another finding was that companies frequently use data waiving based on general and specific rules in the REACH Regulation to adapt the standard testing regime. Evaluation experience showed that justifications were not always adequate. The recently implemented enhanced completeness check includes a manual verification of data waivers to ensure that the justifications either match the provisions set out in Column 2 of REACH Annexes VII-X, or in REACH Annex XI. One of the preliminary outcomes showed that a number of data waivers did not pass a manual verification, and needed either to be refined by the registrant or were replaced by study summaries or read-across[39].

36 https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf/e2e23a98-adb2-4573-b450-cc0dfa7988e5

37 https://www.echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/qsar-models

38 https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

39 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

4.2 ECHA'S ACTIVITIES TO PROMOTE ADEQUATE USE OF ALTERNATIVES AND TO SUPPORT REGISTRANTS

This section summarises the continuous progress on ECHA's commitment both to promote the adequate use of alternatives and to support registrants to comply with their legal duties.

ECHA makes every effort to follow the scientific developments that may mature in Guidance development and updates. ECHA also informs the Commission about possibilities to amend the standard information requirements, as it is important to keep the REACH Annexes up to the scientific and technical development.

4.2.1 Guidance on information requirements and chemical safety assessment

The Guidance has been updated to reflect advancements of the latest scientific developments in the field and ECHA's current best practice[40].

ECHA has further developed the *Guidance on Information Requirements and Chemical Safety Assessment (IR&CSA Guidance)*, in particular several sections of Chapter R.7a related to human health endpoints (i.e. mutagenicity, skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation, reproductive toxicity, acute toxicity, repeated dose toxicity).

One of the main drivers for these updates was to implement recently adopted EU test methods and OECD test guidelines with a potential impact on the replacement or the reduction of animal testing. For some of these endpoints, this can now be achieved by using either standard *in vitro* test methods only (e.g. for skin corrosion/irritation, serious eye damage/eye irritation testing), or *in vivo* methods possibly using less animals than older methods for the same endpoint (e.g. OECD TG 443 Extended One-Generation Reproductive Toxicity Study (EOGRTS) for reproductive toxicity, OECD TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays for mutagenicity).

In its updated IR&CSA Guidance, ECHA has refined the overall testing and assessment strategies for the endpoints discussed above by promoting the use of existing data, weight of evidence, read-across and grouping approaches, to further highlight the "use of animal testing as the last resort" principle. For instance, the updated Section R.7.4 on acute toxicity contains a new weight-of-evidence approach giving the possibility to adapt the REACH Annex VIII standard information requirement for an oral acute toxicity study for non-acutely toxic substances.

Data from an oral repeated dose toxicity study together with other pieces of information coming from *in vitro* cytotoxicity testing, physico-chemical properties, structural analysis and toxicokinetics assessment could be used in certain cases to avoid new oral acute toxicity testing.

Skin sensitisation is an important endpoint for which alternatives test methods have recently become available. ECHA considers the promotion of alternative test methods for this endpoint as a priority and has provided support to an OECD-initiated project to develop guidance documents on the reporting of defined approaches and individual information sources within IATA, which were published in October 2016.

40 https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment and https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

4.2.2 Practical guides

During 2014-2017, ECHA also continued to update practical guides[41]. A new Practical guide for SME managers and REACH coordinators helps registrants to fulfil the information requirements at the 1-10 and 10-100 tpa[42] tonnages.

The Practical guide on how to use alternatives to animal testing to fulfil information requirements for REACH registration[43] was updated. The update made a few individual practical guides obsolete (i.e. *Practical Guide 1: How to report in vitro data; Practical Guide 2: How to report weight of evidence; Practical Guide 4: How to report data waiving; Practical Guide 6: How to report read-across and categories; Practical Guide 10: How to avoid unnecessary testing on animals).*

The practical guide on how to use and report QSARs was also updated[44] to include suggestions on how to assess the validity of QSAR predictions by adding examples for four endpoints, which are relevant for the 2018 registration deadline.

4.2.3 Webinars

Several virtual events[45] have been organised by ECHA on information requirements including topics such as read-across, weight of evidence, *in vitro* data and QSARs. ECHA has hosted four webinars for lead registrants on information requirements.

In September 2016, ECHA held a webinar on the "*Use of alternative methods to animal testing in your REACH registration*"[46]. The webinar focused on recent developments in alternative methods and approaches for skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation and acute toxicity through the dermal route.

The webinar also presented practical examples on what do in different situations depending on the available data.

4.2.4 Read-across assessment framework (RAAF)

The conditions under which read-across and grouping can be used to adapt the standard testing regime are listed in REACH Annex XI, 1.5. Predicting a property based on read-across must be reliable, can be used for risk assessment and/or classification and labelling, and complies in general with the provisions in REACH for the substance under consideration.

41 https://echa.europa.eu/practical-guides

42 https://echa.europa.eu/documents/10162/13655/pg_sme_managers_reach_coordinators_en.pdf/1253d9f9-d1f0-4ca8-9e7a-c81e337e3a7d

43 https://echa.europa.eu/documents/10162/13655/practical_guide_how_to_use_alternatives_en.pdf/148b30c7-c186-463c-a898-522a888a4404

44 https://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf/407dff11-aa4a-4eef-a1ce-9300f8460099

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

46 https://echa.europa.eu/view-webinar/-/journal_content/56_INSTANCE_DdN5/title/use-of-alternative-methods-to-animal-testing-in-your-reach-registration

Methods for building read-across cases are already described in ECHA Guidance[47] and the *Practical guide on how to use alternatives to animal testing to fulfil your information requirements for REACH registration*[48].

ECHA has codified a systematic approach to assess read-across cases that are encountered in its dossier evaluation activities. This systematic approach is called "The Read-Across Assessment Framework", or RAAF[49] and provides a framework and guidance to consistently evaluate the scientific aspects of a proposed read-across case, resulting in an output, which is suitable for subsequent regulatory consideration of the read-across case.

In developing this approach, ECHA also sought to accommodate a wide range of views and expertise from stakeholders at workshops held in 2012 and 2014.

ECHA published the RAAF for human health endpoints in 2015. An update of the document was published in February 2017, addressing environmental endpoints. A further document describing the key issues relevant for specifically addressing multi-constituent substances and UVCBs was published in March 2017[50].

The RAAF is primarily designed for use by ECHA's experts to consistently assess read-across encountered during dossier evaluation. However, the publication also gives an insight for registrants on how to assess, and improve where they can, their explanations of why and how read-across can be used in their adaptations.

Most of the encountered weaknesses of the read-across cases may be avoided by applying the following checklist:

- Make use of ECHA's Read–Across Assessment Framework to check the robustness of your read-across adaptation.
- Give a hypothesis-driven justification why the data from one substance can be used to fill the data gap for another substance. Do that for each property.
- Analyse experimental data for contradictions against the proposed hypothesis. Justify read-across adequately and provide supporting and credible information.
- Specify the identity of all substances used. Consider also impurities and potentially different substance compositions when developing a read-across argument.
- Show how structural similarity and dissimilarity justify the prediction.
- Create a data matrix, highlighting trends within the category.

An important step for improving the acceptability is that read-across should be seen as being endpoint-specific. It is highly recommended that the hypothesis and supporting evidence are specific for the given endpoint, potentially addressing the endpoint-specific nature of the mechanism of action.

47 https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

48 https://echa.europa.eu/documents/10162/13655/practical_guide_how_to_use_alternatives_en.pdf/148b30c7-c186-463c-a898-522a888a4404

49 https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

50 https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

4.2.5 OECD QSAR Toolbox project

The OECD QSAR Toolbox[51] is a software that can be used to group chemicals into categories and help fill gaps in (eco)toxicity data, which is needed to assess the hazards of chemicals.

The tools that are integrated in the Toolbox consist of chemical inventories and toxicological databases, as well as statistical tools for mining knowledge from existing data. It also includes 'profilers' that are compilations of structural features and decision rules and some QSAR models. These profilers can be used in various different ways. However, they were found to be particularly helpful when identifying mechanistic similarities between substances for read-across purposes.

The QSAR Toolbox is continuously developed taking into account the feedback received from users. Version 4.0, released in April 2017, is more user-friendly, particularly for less experienced users. The prediction process for lower tier endpoints (automated/standardised workflows for skin sensitisation and acute aquatic fish toxicity in V4.0) is more streamlined. More comprehensive tools (e.g. mapped observed metabolism) supporting formations of chemical categories for high tier endpoints have also been added. It has to be noted that predictions made with the QSAR Toolbox should be scientifically supported and appropriately documented.

There is a lot of training material available from the website cited above. A valuable source of knowledge are the endpoint-specific training materials. Furthermore, ECHA developed its own case studies, which are meant to serve as examples for good practices when using the QSAR Toolbox[52].

The OECD QSAR Toolbox will be further developed and improved before the third REACH registration deadline in 2018, and beyond. This is in line with the OECD focus on this methodology in the next 5 to 10 years.

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.

4.3 ECHA'S ACTIVITIES TO PROMOTE THE DEVELOPMENT OF SUITABLE ALTERNATIVES

Especially for high tier endpoints, it is apparent that neither *in vitro* methods nor QSARs will be able to replace animal testing in a simple one-to-to manner for complex hazard endpoints. Read-across is a promising methodology for high tier endpoints, although ECHA's evaluation experience was that read-across was often not substantiated with thorough argumentation. Furthermore, supporting evidence was frequently lacking. One of ECHA's priorities is therefore to promote the development of methodologies which could potentially support companies to get a better understanding of the toxicology of high tier human health and environmental endpoints and which may provide the necessary evidence to support an argumentation.

In the section below, ECHA's activities to promote the development of alternatives is explained in more detail.

51 http://www.qsartoolbox.org/

52 http://echa.europa.eu/web/guest/support/oecd-qsar-toolbox

4.3.1 Data to support alternatives

Since 24 March 2017, ECHA is making data - submitted by registrants to meet REACH requirements - available for download with the aim of increasing its use and improving the safe use of chemicals worldwide as well as supporting the use and development of alternative methods[53].

The data are available in IUCLID 6 format and can be imported, read and searched with the IUCLID application. It includes specific parts of the information published online, respecting the ownership rights of companies who submitted the data. For example, the downloadable dataset includes the results from studies conducted by companies, but it does not provide the full study summary. In addition, the material does not include the data companies have claimed confidential in their registrations.

The available data can be used to develop new ways of determining the toxicity of chemicals with the aim of minimising the need for testing on animals.

4.3.2 Steering scientific priorities

One of the priority areas for ECHA's regulatory science activities are non-animal alternative methods and new approaches to hazard assessment, in particular rational integration of different lines of evidence and other means of reduction or refinement when non-animal approaches are not yet available[54].

There is a wide range of methods available to perform hazard assessments of chemicals, including "traditional" toxicological studies, *in vitro* tests, "read-across"/"chemical categories", QSARs, and "high throughput screening" approaches. Research is needed to combine these approaches, perhaps into integrated testing strategies or similar. In addition, to support such combined approaches, further fundamental research will be necessary about the biological mechanisms that underpin toxicity or ecotoxicity. The mechanistic understanding of involved pathways of toxicity can be facilitated by applying the adverse outcome pathway (AOP) concept.

ECHA is monitoring the progress in current developments as it may impact judgements about the scientific adequacy of information provided by companies, regulatory opinions and decisions, or guidance about how to fulfil the requirements of the legislation. Examples of scientific developments include effects of a chemical on the endocrine system of humans and wildlife, hazards and risks posed by nanomaterials, and the combination of effects of chemicals.

For some low tier human health endpoints, alternative test methods are available, or will become available soon. Further adaptation possibilities for data waiving have also been incorporated into REACH (e.g. for acute dermal toxicity). However, there is no alternative yet to replace the 28-day repeated dose toxicity and reproductive toxicity screening studies. In addition to being a standard information requirement of Annex VIII, such studies have been used as bridging (supporting) studies by registrants as part of testing programmes based on a read-across approach involving registrations at 100 tpa or more. For high tier toxicological testing, more scientific development is needed before they can be replaced by alternatives.

Many of the testing methods described in this report are already available to registrants. There are also new methods, which are still in research and development. These methods may not be necessarily suitable in a regulatory context for satisfying the requirements of REACH (e.g. for one-to-one replacement of a standard test). Registrants are advised to be aware of the limitations of such methods depending on the

53 https://iuclid6.echa.europa.eu/reach-study-results

54 https://echa.europa.eu/documents/10162/13609/echa_science_strategy_final_web_en.pdf

case in question. A challenge is how to understand which of these techniques will be suitable for regulatory purposes.

Further developments in integrated testing strategies (ITSs) and integrated approaches to testing and assessment (IATAs)[55] look promising, as well as adverse outcome pathway (AOP)-based approaches[56] for predicting hazard in the long term.

In 2016, ECHA hosted a topical scientific workshop on new approach methodologies (NAMs)[57]. NAMs were addressed in a broad context to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as including information from the exposure of chemicals in the context of hazard assessment.

NAMs also include a variety of new testing tools, such as the "high-throughput screening" and "high-content methods" e.g. genomics, proteomics, metabolomics; as well as some "conventional" methods aiming to improve the understanding of toxic effects, either through improving toxicokinetic or toxicodynamic knowledge. The workshop discussed three specific aspects of NAM application:

1) the potential to support the read-across hypothesis and justification,

2) the way they provide an input for screening and prioritisation of substances, and

3) prospects for regulatory science.

Specific emphasis was given to the US ToxCast programme for endocrine disrupting chemicals and metabolomics as presented in a case of read-across for herbicides. The US Tox21 vision was also presented. In this workshop, but also in publications like the Joint Research Centre's (JRC) report on 3Rs knowledge sharing[58], the Accelerating the Pace of Chemical Risk Assessment (APCRA) workshop introduction[59], and the OECD's IATA case study project considerations[60], it is apparent that scientific development work in this area would benefit from a more regulatory focus to ensure safe use.

4.3.3 Specialised workshops and other activities

In addition to the conferences and events of general interest, ECHA organises expert workshops for specialised audiences to gain insight and feedback from industry on specific areas, like workshops on the OECD QSAR Toolbox.

In April 2015, ECHA hosted an EPAA – Cefic – Cosmetics Europe cross sector workshop on "*Alternatives for skin sensitisation – Hazard identification and potency categorisation*". The aim was to bring industry and

55 https://echa.europa.eu/documents/10162/13609/echa_science_strategy_final_web_en.pdf

56 https://echa.europa.eu/view-article/-/journal_content/title/topical-scientific-workshop-new-approach-methodologies-in-regulatory-science

57 https://echa.europa.eu/view-article/-/journal_content/title/topical-scientific-workshop-new-approach-methodologies-in-regulatory-science

58 https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/accelerating-progress-replacement-reduction-and-refinement-animal-testing-through-better

59 Daily Environment Report,223 DEN B-1, 11/18/16. Copyright 2016 by The Bureau of National Affairs, Inc. (800-372-1033) http://www.bna.com

60 OECD (2016) Report on Considerations from Case Studies on Integrated Approaches for Testing And Assessment (IATA), First Review Cycle (2015), Case Studies on Grouping Methods as a Part of IATA, No. 250, Series on Testing & Assessment. ENV/JM/MONO(2016)48, OECD, Paris; http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)48&doclanguage=en

regulators together and to discuss the use of alternative test methods that are currently under validation or have been adopted for the skin sensitisation endpoint. More information can be found in the published report[61] and in a peer-reviewed publication[62].

Alternative methods covering 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. Neither are they considered acceptable for the purposes of fulfilling the respective standard information requirements. The use and promotion of read-across, with a focus on repeated dose toxicity, is a priority for ECHA's contribution to the EU-ToxRisk project[63], which aims to integrate the latest scientific developments with current regulatory practice.

ECHA supports a number of scientific and regulatory activities through the OECD or through a number of bilateral agreements with international partners including regulatory bodies in Australia, Canada, Japan and the USA.

ECHA is already collaborating with the JRC and aims to further develop this cooperation to both influence and benefit from the latest scientific developments.

61 http://cefic-lri.org/wp-content/uploads/2014/03/Joint-WS-Skin-Sensitization-Alternatives-2015-Flash-report.pdf

62 Alternatives for skin sensitisation: Hazard identification and potency categorisation: Report from an EPAA/Cefic LRI/Cosmetics Europe cross sector workshop, ECHA Helsinki, April 23rd and 24th 2015, Basketter et al (2015) Regulatory Toxicology and Pharmacology, Volume 73, Issue 2, Pages 660–666

63 www.eu-toxrisk.eu/

5. CONCLUSIONS

In general, data sharing and the joint submission of information has worked well based on the high number of registrations submitted jointly and how registrants have used them to efficiently fulfil their information requirements. This conclusion is also in line with the findings in the previous report in this series[64].

In addition, registrants have overall made extensive use of existing information (old experimental studies) and adaptation possibilities before conducting new studies or proposing new high tier vertebrate animal tests. More experimental data are available for low tier human health endpoints compared to high tier human health and environmental endpoints, for which in the absence of experimental data greater use was made of the adaptation possibilities provided under REACH.

Taking all of the different computational analyses together, the consistent finding is that the use of read-across is the key alternative approach found in the registration dossiers. Read-across is considered one of the main adaptations possible for high tier human health endpoints such as repeated dose toxicity, developmental and reproductive toxicity, presuming that a scientifically plausible hypothesis can be justified and used for deriving a quantitative result for the targeted substances.

Experience from evaluation indicates that such adaptations are often found to be inadequate to safeguard the safe use of chemicals. In such cases, ECHA requests the missing information, including animal tests where these are necessary to fulfil the information requirements.

Where registrants considered that adapting the high tier information requirements was not possible, they have to submit a testing proposal to ECHA and await its decision on the testing. The available information so far shows that the vast majority of testing proposals that were examined were considered necessary and resulted in an adopted decision.

Weight of evidence is used less frequently than read-across as the principal option. The weight-of-evidence approaches mainly comprised the use of read-across and old experimental studies. There may be benefits in further developing advice and guidance for this adaptation of how to assess the quality of individual documents and the uncertainties arising when combining different sources of information to adapt an information requirement under REACH. ECHA is working in this direction.

Data waiving was used a lot, especially for high tier environmental endpoints. This is partially due to the tiered or conditional information requirements of REACH, meaning the need to fulfil an information requirement on an endpoint depends on the properties of, or the exposure to, the substance. Experience from evaluation showed that justifications for data waiving were not always adequate. The recently implemented enhanced completeness check therefore includes a manual verification of data waivers to ensure that the justifications either match the provisions set out in Column 2 of REACH Annexes VII-X, or in REACH Annex XI[65].

Registrants already use existing alternative methods and approaches for skin corrosion/irritation and serious eye damage/eye irritation. In some cases, the information requirement was fulfilled by using *in vitro* test data either alone or together with other information, mainly existing *in vivo* studies with the registered substance or applied read-across approach (about 20 %).

64 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=animal-testing-reports#animal-testing-reports

65 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

However, registrants largely make use of existing *in vivo* studies only (>50 %), and to a lesser degree new *in vivo* studies only (up to about 4 %) to fulfil the requirements. In some cases of new *in vivo* tests, the reasons why the tests were conducted is not always clear from the information given in the study summary.

Initial findings from these cases suggest that the majority were performed for other regulatory purposes (such as other regulatory requirements than REACH). ECHA has provided tools and practical guides on how to properly use newly developed methods and to build good quality QSAR predictions.

In respect of the information requirement for skin sensitisation, registrants have made use of information obtained by applying read-across strategies as well as non-animal test batteries to avoid animal testing. However, non-animal test methods and the associated testing strategy have only recently been implemented in REACH. Hence, when comparing the use of *in chemico/in vitro* studies in the dossiers analysed for the previous report and this one, the use of such methods has increased only slightly.

However, some *in vivo* tests may be available for other reasons. For example, if registrants under REACH obtain access to such studies as they were conducted to fulfil regulatory requirements outside the EU. If ECHA suspects that a registrant has not complied with their obligations under REACH to use alternative methods, Member State authorities can be informed of this for consideration of any enforcement action.

Due to the increasing number of registrations, the absolute numbers of new experimental studies are naturally increasing for all endpoints. However, particularly for high tier endpoints, the numbers for new studies are not as high as might have been expected from the number of substances registered. Therefore, while registrants make use of full provisions under REACH to avoid unnecessary animal testing, it may mean that, at the same time, there is insufficient data to properly identify their long-term hazardous properties.

The findings from the analysis in this report of the extensive use of adaptations, particularly for high tier endpoints, when taken together with deficiencies identified in their use, will be used to help ECHA further refine its efforts to promote the proper use of alternative methods and to support further scientific development. As was mentioned before, to support proper use of adaptations for high tier human health and environmental endpoints, additional supporting evidence related to mechanisms of toxicity or toxicokinetics will often need to be generated.

In this respect, ECHA sees potential in new approach methodologies (NAMs) in the longer term, as these methods are based on models able to detect specific mechanisms of toxicity, provide kinetic information and be run in a high-throughput manner. Therefore, ECHA will follow and support the scientific developments of methods that could ultimately limit or replace the need for new studies in animals in the long term. A priority are those methods, which show promise in the support of read-across.

Finally, ECHA will continue in its efforts to promote the use of alternatives through its publications, website, guidance development, campaigns, events and the downloadable dataset. In the context of the reduction, refinement and replacement of animal testing (3Rs) principles, ECHA is also planning to publish a state-of-the-art review on the availability and regulatory applicability of alternative and non-animal approaches later in 2017.

APPENDICES

Appendix 1: Details on the methodology

For this report, any option given to the registrant that does not use experimental studies on vertebrate animals sufficient on their own to fulfil the standard information requirement, is counted as an adaptation.

The analysis focused on the following types of adaptations: read-across, QSARs, weight of evidence, *in vitro* tests and data waiving.

In vitro methods are analysed separately because for a limited number of endpoints there are already regulatory accepted *in vitro* alternatives available.

Although not an alternative method, data waiving was also counted as an adaptation because they can exclude the need for new animal studies. The use of all these types of adaptations is compared to the use of experimental data on vertebrate animals.

A difference to the previous report is that the current edition does not analyse information submitted in a category template separately anymore. To make the picture as complete as possible, the dossiers with a category template have been analysed together with all other dossiers. The category template was used for handling information on groups of substances. It helped the registrant to fill the IUCLID dossier by providing group information together.

To comply with the REACH regulation, the registrant had to submit one dossier for each substance. In this dossier, the read-across is justified per substance and per endpoint to meet the information requirements for a substance. Thus, there was no need to separate the category template from other read-across records and for this report all read-across records were counted in an equal manner, irrespective of whether a category template in IUCLID was used or not.

In line with the analyses performed in the first and second reports, no estimation of the number of vertebrate animals saved by the use of alternative approaches in REACH was conducted, due to the significant number of (estimated) assumptions that would be required to perform such an analysis.

To keep a level of consistency and comparability between this and the previous reports in this series, a similar approach for data analysis and data presentation was followed.

Endpoint study records (ESRs) formed the basis for the computational analysis. These are specific entries filled by registrants for the hazard endpoints in the IUCLID dossiers. For each study, an ESR has to be created under the given endpoint. Registrants can attempt to fulfil an endpoint information requirement by using multiple records. If, for instance, multiple studies are available for the same endpoint, these result in multiple records.

The information requirements for a given endpoint can also be addressed by different types of data (e.g. experimental data, read-across, and QSAR). Often when applying REACH Annex XI options, registrants have used combinations of these, with or without studies. As a result, many information requirements are fulfilled by the registrants with a combination of information.

The Agency has developed an IT algorithm (set of workflows) allowing for the assignment of labels to each ESR as follows:

- Assignment as an old or new study: all ESRs with a purpose flag that equals “experimental result” have been labelled as follows:

  - old study: for ESRs with a reference year < 2009;
  - new study: for ESRs with a reference year >= 2009;
  - If there is conflicting information in the reference table for the same study (multiple reference years pointing to years before and after 2009), the ESRs were labelled as an old study because of the observations that registrants did not always provide the correct year of the study, but the year when they obtained access to it.

- Assignment of REACH Annex according to which information requirements applied (REACH Annexes VII – X):

  - for tonnages more than 1 000 tpa assign: "Annex X";
  - for tonnages 100-1 000 tpa assign: "Annex IX";
  - for tonnages 10-100 tpa assign: "Annex VIII";
  - for tonnages <10 tpa assign: "Annex VII".

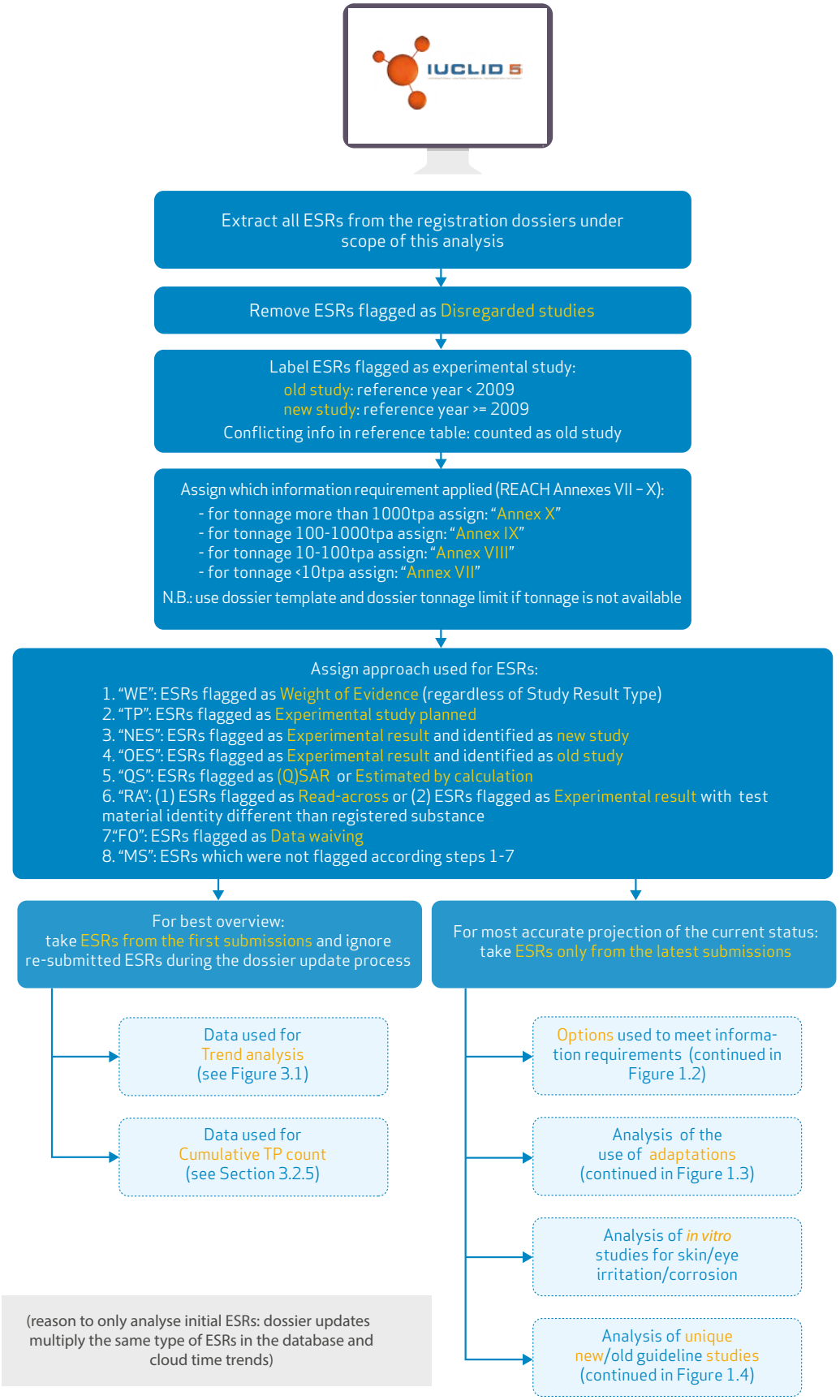
- Assignment of ESR types:

  - "WE": ESRs flagged as weight of evidence (regardless of the study result type);
  - "TP": ESRs flagged as experimental study planned;
  - "NES": ESRs flagged as experimental result and identified as new study;
  - "OES": ESRs flagged as experimental result and identified as old study;
  - "QS": ESRs flagged as QSAR or estimated by calculation;
  - "RA": (a) ESRs flagged as read-across or (b) ESRs flagged as Experimental result with test material identity different than registered substance;
  - "FO": ESRs flagged as data waiving (flags to omit study);
  - "MS": ESRs which were not flagged as one of the above.

Note that the terminology between IUCLID 5 and IUCLID 6 changed. For example 'Study result type' is called 'Type of information', 'Purpose flag' is called 'Adequacy of study' under IUCLID 6, respectively.

For this report, which focuses on vertebrate animal testing, ESRs referring to invertebrates were only excluded from the analysis for bioaccumulation (27 % of ESRs submitted for bioaccumulation refer to invertebrates; such ESRs referring only to invertebrates were submitted for 7 % of the substances for which bioaccumulation information was reported; less than 1 % of the substances were found to be covered by ESRs referring to invertebrates only).

Figure 1.1: Schema illustrating the computational analysis of the endpoint study records. An oval links to a further process.



1.1 OPTIONS USED TO ADDRESS INFORMATION REQUIREMENTS (SUBSTANCE PROJECTION)

As in the previous report, it was of interest to analyse how the registrants used different options to meet information requirements (substance projection in previous reports). In doing so, a hierarchy in counting was applied. This means that the options were counted in some order.

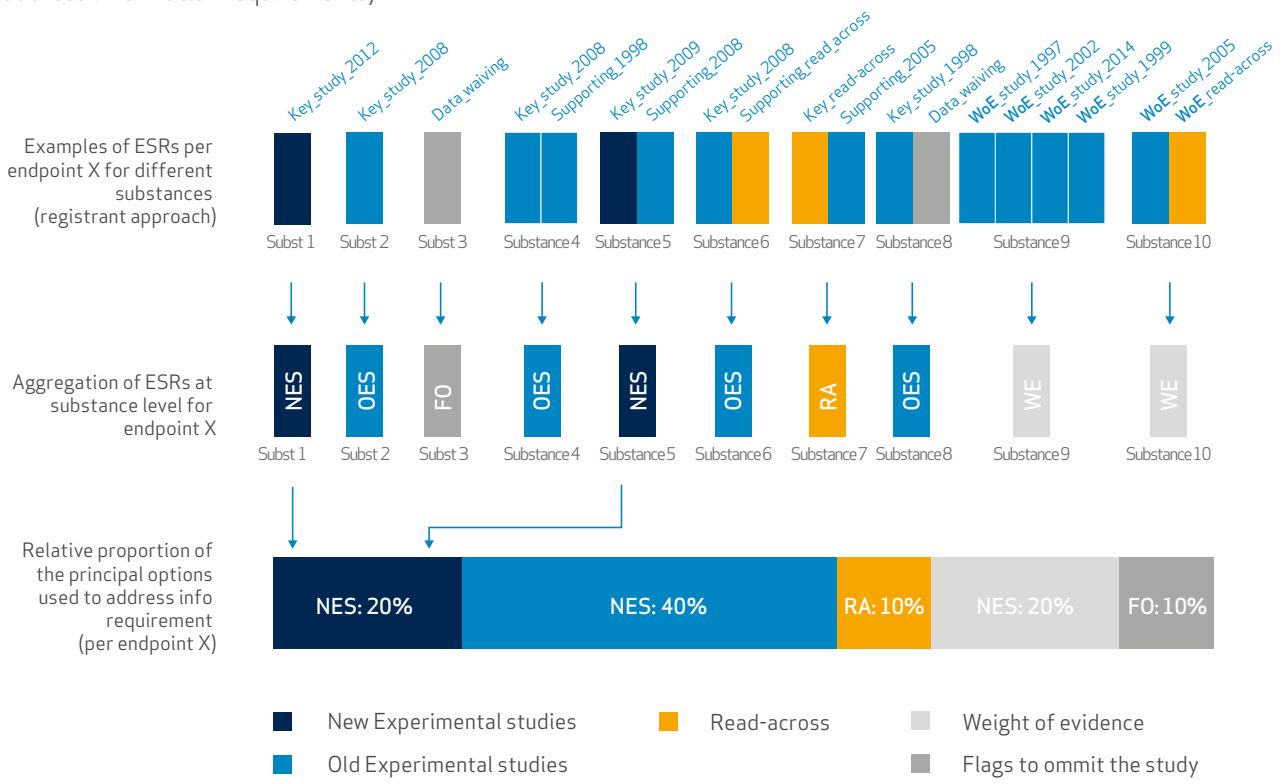
The order in which the assignment of the main options have been applied is explained under the header "Analysis of options used to address information requirements", in Section 3.1. The order is: weight of evidence (WE), testing proposal (TP), new experimental study (NES), old experimental study (OES), QSAR (QS), read-across (RA), flags to omit the study (FO), and miscellaneous (MS).

Figure 1.2 illustrates the choices that could have been made for combining different options in this substance projection.

In this analysis, the experimental studies available for each substance have not been checked for the study outcome or for the quality of the information. Quality of information is checked during the dossier evaluation process and the outcomes are described in ECHA's evaluation reports[66].

Therefore, it is important to note here that an entry as an experimental study under an endpoint does not necessarily mean that the information requirement has been fulfilled according to the requirements in the REACH annexes.

Figure 1.2: Illustration on how multiple ESRs are converted into the substances in this projection (options used to address information requirements)



66 https://echa.europa.eu/about-us/the-way-we-work/plans-and-reports?panel=evaluation-reports#evaluation-reports

1.2 USE OF ADAPTATIONS TO THE STANDARD INFORMATION REQUIREMENTS

This analysis has been developed to complement the main analysis described above and provides a full overview of the adaptations as they have been used by registrants. It focuses on adaptations mentioned in the REACH Annex XI: read-across, QSARs, weight of evidence and data waiving. In this analysis, there was no distinction between data waiving triggered by specific Column 2 adaptations foreseen in REACH Annexes VII to X or general REACH Annex XI provisions, because the net result was reducing the number of tests and the use of vertebrate animals. All adaptations (regardless of any other option reported for this substance) are counted without any additional conditions. This means that more than one adaptation can be counted per substance and endpoint.

The detailed results from analysing the adaptations to the standard testing regime are presented in Appendix 4. Figure 1.3 illustrates how data were aggregated and processed in this projection. Further analysis of different adaptations were performed specifically to each adaptation, e.g. it was interesting to know if read-across and QSAR are used as a key or supporting study, if weight of evidence was based on experimental data or included other alternatives as well, if the arguments for omitting studies were based on scientific or technical reasons, or if it is exposure-based waiving.

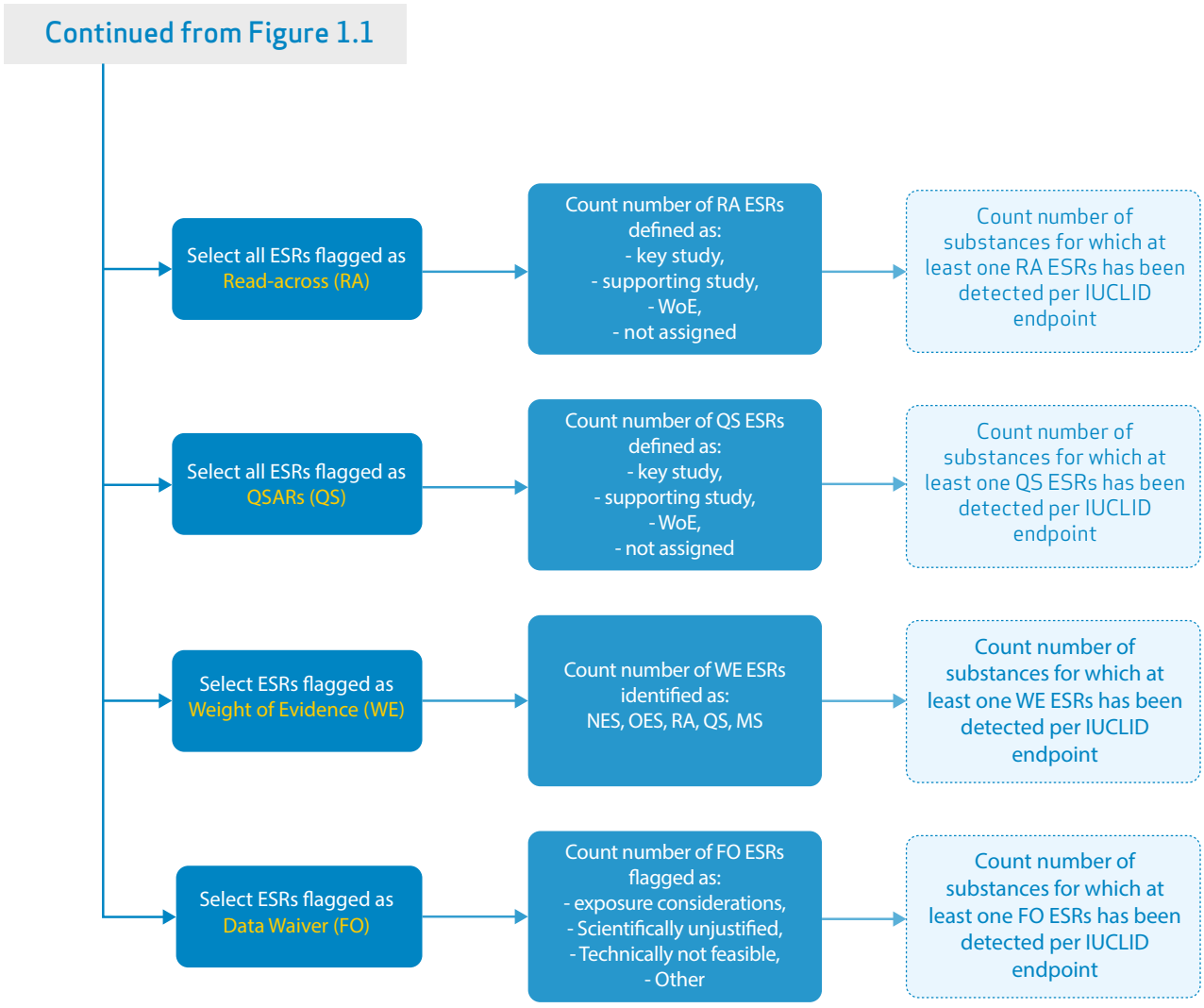
1.3 ANALYSIS OF NON-ANIMAL TEST METHODS FOR SKIN CORROSION/IRRITATION, SERIOUS EYE DAMAGE/EYE IRRITATION AND SKIN SENSITISATION

Endpoints for which non-animal test methods have been developed and revised are skin corrosion/irritation, serious eye damage/eye irritation, and skin sensitisation. These endpoints were selected for analysis in more detail.

These test methods, if used within the limitations of their applicability, can be used to generate information to fulfil the REACH Annex VII information requirements for *in vitro/in chemico* tests either alone or in a testing and assessment strategy. Furthermore, depending on the test methods used and on the outcomes of these tests, the resulting information may allow for conclusions on classification and risk assessment based solely on such test methods, without the need to perform an *in vivo* study (for substances falling under the REACH Annex VII and VIII information requirements).

The numbers of *in vitro* and *in vivo* studies for the above-mentioned endpoints were checked manually. Attention was paid in particular to the general trends of use of *in vitro* methods by registrants and especially when registrants use the *in vitro* methods as information alone to fulfil their information requirements.

Figure 1.3: Analysis of adaptations to the standard information requirements. Continue from Figure 1.1. Legend: RA – read-across; QS – QSAR; WE – weight of evidence; FO – flags to omit study



1.4 NEW AND OLD EXPERIMENTAL STUDIES CONDUCTED ACCORDING TO FORMALLY ADOPTED TEST GUIDELINES

The workflow for counting unique studies conducted under respective test guidelines is shown in Figure 1.4. The unique experimental study (UES) concept was introduced since the same ESRs (i.e. referring to identical studies) might have been used in several dossiers, especially if read-across or category of substances is concerned. Hence, by applying a unique identifier concept, it is possible to avoid multiple counting of the same study.

The UES algorithm has been optimised to spot duplicates in experimental results, also across endpoints (e.g. combined repeated dose toxicity/reproductive screening studies).

Only studies declared by the registrant as having a good quality (i.e. quality of Klimisch scores 1 and 2) were considered.

The algorithm for generating the unique guideline study results covers the following steps:

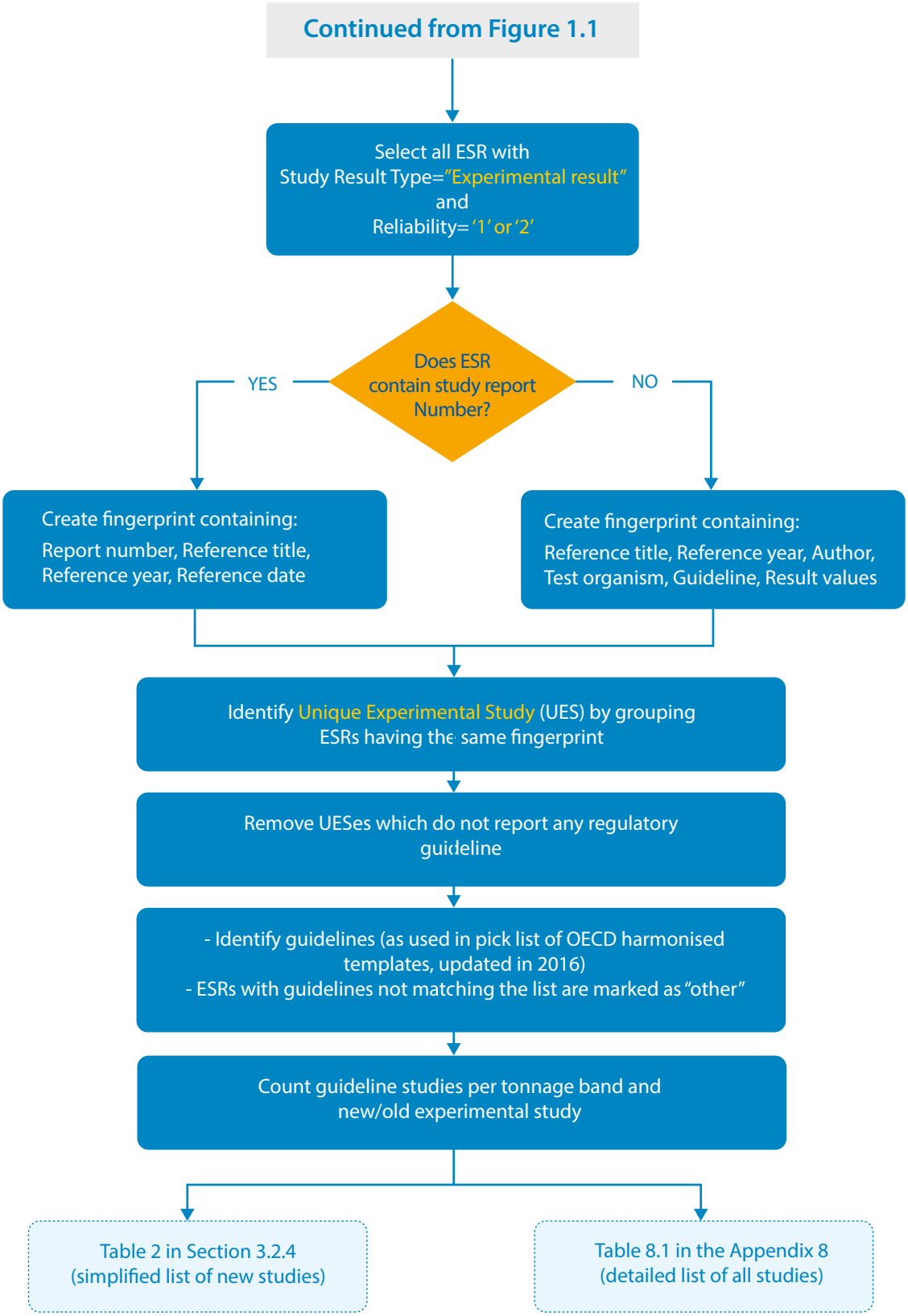
- Select ESRs with study result type equals "experimental result" and Klimisch score 1 and 2;
- For each ESR, create the fingerprint as follows:
    - If the registrant filled the "report number" field in the study reference, the fingerprint contains: report number, reference title, reference year and reference date;
    - Otherwise the fingerprint contains: reference title, reference year, author, test organism, guideline and study result values;
- Create UES by removing multiple ESRs with identical fingerprints;
- Remove UES, which do not report any guideline;
- Identify the guideline studies by mapping the reported guidelines versus the study list defined in the OECD harmonised template (OHT) for the endpoint of interest (OECD, 2016[67]).

A manual verification between the data mining algorithm results and registration dossier information was performed for skin corrosion/irritation and serious eye damage/eye irritation.

New experimental studies (i.e. with a reference date of 2009 or later) are presented in Table 2 of the main report. All unique experimental studies listed by test guideline are presented in Appendix 8 (data availability table) and they are not commented in detail in the report.

67 https://www.oecd.org/ehs/templates/

Figure 1.4: Workflow for identifying unique new and old experimental studies



1.5 TESTING PROPOSALS

Testing proposals, as submitted originally by registrants can change over time (e.g. a testing proposal might be withdrawn, or a proposal can be replaced by a test result). In addition, the final outcome of a testing proposal will not be known until after the deadline for submitting any required information expires, which in a number of cases is several years away.

To provide an overview of the fate of submitted testing proposals, analyses at different stages in the life cycle of the testing proposals have been conducted. These stages are data submission, dossier evaluation and follow-up to dossier evaluation, and are described in detail in Section 3.1.

1.6 TREND ANALYSIS

For the trend analysis, only the initial ESRs were counted, meaning that repeated submissions of the same ESR (identified through a fingerprint approach using key fields in the ESR) in an updated dossier were removed from the initial data pool. The dossier updates multiply the same ESRs in the database because once a dossier is updated (for example, due to a tonnage update or a legal entity change), the same ESRs are re-submitted in the updated dossier. If they are not removed, they would be counted multiple times and thus trends of using one or another option to address information requirements would be overloaded with previously submitted ESRs.

The fingerprints were created using information from the following sections of the ESRs:

- IUCLID endpoint;
- Study period;
- Reference report date;
- Reference author;
- Reference year;
- Reference title;
- Test guideline and text;
- Approach used.

To avoid peaks around the deadlines, the ESRs were normalised by the total number of dossiers submitted per year. The ESRs were counted for each IUCLID section (translated to endpoint), phase-in status, REACH Annex (VII - X), adaptation approach, and year.

The results of this approach show also, what information have been submitted for a given endpoint cumulatively in all dossiers. However, it does not cover which data have been used as key data to fulfil the information requirements. The type of use (e.g. key or supporting study) for the most recent registration dossiers have been analysed in the REACH Annex XI projection (Appendix 4).

Appendix 2. Detailed results of the analysed endopoint study records

Methodology for preparing IUCLID dossiers for analysing the endpoint study records (ESRs) is described in Appendix 1.

Table 2.1: Overview table with number of ESRs per endpoint and option type. NES – new experimental studies; WE – weight of evidence; RA – read-across; QS – QSAR; testing proposal – testing proposal; FO – flags to omit study; MS – miscellaneous.

NUMBER OF ESRs PER OPTION AND PER ENDPOINT

IUCLID Section	NES	OES	TP	RA	QS	WE	FO	MS	Total
Bioaccumulation	137	839	14	1 448	2 462	4 030	3 298	322	12 550
Short-term toxicity to fish	1 221	8 537	0	6 850	941	3 476	327	1 016	22 368
Long-term toxicity to fish	153	966	29	3 040	463	1 199	3 681	200	9 731
Toxicity to birds	21	467	0	662	3	1 060	2 977	51	5 241
Toxicokinetics	495	5 026	4	5 440	242	2 932	730	1 605	16 474
Acute toxicity (oral)	1 083	14 325	0	5 682	139	2 055	361	642	24 287
Acute toxicity (inhalation)	375	7 608	0	2 941	94	1 451	2 887	535	15 891
Acute toxicity (dermal)	788	8 742	0	2 696	60	994	1 432	319	15 031
Skin irritation/ corrosion	1 885	18 558	0	5 176	170	2 373	802	714	29 678
Eye irritation	1 835	11 317	0	4 419	129	1 842	856	692	21 090
Skin sensitisation	1 473	9 088	0	3 852	197	2 558	784	793	18 745
Repeated dose toxicity (oral)	1 375	5 739	304	7 109	81	2 862	1 777	379	19 626
Repeated dose toxicity (inhalation)	335	4 386	34	3 191	92	551	2 641	278	11 508
Repeated dose toxicity (dermal)	34	6 270	5	838	3	170	3 095	184	10 599
Genetic toxicity *in vitro*	3 592	14 859	0	10 405	279	5 959	883	1 675	37 652
Genetic toxicity *in vivo*	446	4 817	60	3 621	36	1 428	421	416	11 245
Carcinogenicity	118	3 546	0	2 893	90	892	1 077	842	9 458
Reproductive toxicity	1 127	2 137	251	3 747	122	2 124	3 494	209	13 211
Developmental toxicity/ teratogenicity	828	4 334	460	5 238	101	2 292	2 175	265	15 693
Total	**17 321**	**131 561**	**1 161**	**79 248**	**5 704**	**40 248**	**33 698**	**11 137**	**320 078**

Table 2.2 Number of ESRs per endpoint, phase-in status, REACH Annex and option type. NES – new experimental studies; WE – weight of evidence; RA – read-across; QS – QSAR; testing proposal – testing proposal; FO – flags to omit study; MS – miscellaneous

Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Bioaccumulation	Non-phase In	Annex X	3	7	0	12	106	71	65	5	
		Annex IX	4	37	1	45	37	63	125	0	
		Annex VIII	8	38	1	10	70	85	39	4	
		Annex VII	3	18	0	3	6	43	9	0	
	Phase In	Annex X	68	385	5	546	1 478	1 008	1 462	227	
		Annex IX	39	231	7	497	499	2 091	1 303	57	
		Annex VIII	8	57	0	243	146	531	176	15	
		Annex VII	4	66	0	92	120	138	119	14	
Total			**137**	**839**	**14**	**1 448**	**2 462**	**4 030**	**3 298**	**322**	**12 550**
Short-term toxicity to fish	Non-phase In	Annex X	26	102	0	95	41	91	6	12	
		Annex IX	29	160	0	142	1	18	7	7	
		Annex VIII	105	340	0	74	25	28	16	21	
		Annex VII	108	177	0	27	7	1	5	9	
	Phase In	Annex X	348	5 360	0	2 583	617	1 156	124	735	
		Annex IX	464	1 563	0	2 684	136	1 548	141	147	
		Annex VIII	108	305	0	847	41	520	21	19	
		Annex VII	33	530	0	398	73	114	7	66	
Total			**1 221**	**8 537**	**0**	**6 850**	**941**	**3 476**	**327**	**1 016**	**22 368**
Long-term toxicity to fish	Non-phase In	Annex X	10	4	0	19	16	68	69	2	
		Annex IX	16	16	0	42	6	4	151	0	
		Annex VIII	10	15	0	10	1	0	28	1	
		Annex VII	4	4	0	3	1	1	4	0	
	Phase In	Annex X	63	576	6	1 097	330	448	1 482	138	
		Annex IX	32	219	22	1 288	70	608	1 638	47	
		Annex VIII	12	38	1	470	28	45	216	6	
		Annex VII	6	94	0	111	11	25	93	6	
Total			**153**	**966**	**29**	**3 040**	**463**	**1 199**	**3 681**	**200**	**9 731**
Lond-term toxicity to birds	Non-phase In	Annex X	1	0	0	25	0	32	97	0	
		Annex IX	0	6	0	10	0	3	33	0	
		Annex VIII	9	27	0	0	0	13	9	0	
		Annex VII	0	5	0	0	0	1	2	1	
	Phase In	Annex X	6	313	0	253	1	290	2 033	29	
		Annex IX	1	94	0	255	2	583	633	17	
		Annex VIII	1	15	0	110	0	113	107	4	
		Annex VII	3	7	0	9	0	25	63	0	
Total			**21**	**467**	**0**	**662**	**3**	**1 060**	**2 977**	**51**	**5 241**

Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Toxicokinetics	Non-phase In	Annex X	12	23	0	91	8	60	19	13	
		Annex IX	23	64	0	118	5	29	31	48	
		Annex VIII	26	71	0	35	23	43	38	112	
		Annex VII	5	16	0	4	4	3	2	37	
	Phase In	Annex X	220	3 058	4	2 803	75	1 199	390	669	
		Annex IX	157	1 168	0	1 855	108	1 241	209	546	
		Annex VIII	43	415	0	312	11	285	24	112	
		Annex VII	9	211	0	222	8	72	17	68	
Total			**495**	**5 026**	**4**	**5 440**	**242**	**2 932**	**730**	**1 605**	**16 474**
Acute toxicity (oral)	Non-phase In	Annex X	20	170	0	119	0	76	10	7	
		Annex IX	30	151	0	105	0	21	8	6	
		Annex VIII	80	314	0	55	1	16	7	16	
		Annex VII	281	458	0	100	1	41	35	10	
	Phase In	Annex X	176	9 336	0	2 118	53	719	162	463	
		Annex IX	328	2 493	0	2 067	40	756	84	88	
		Annex VIII	70	623	0	752	15	309	20	25	
		Annex VII	98	780	0	366	29	117	35	27	
Total			**1 083**	**14 325**	**0**	**5 682**	**139**	**2 055**	**361**	**642**	**24 287**
Acute toxicity (inhalation)	Non-phase In	Annex X	14	98	0	101	0	49	61	9	
		Annex IX	12	37	0	61	0	21	137	8	
		Annex VIII	21	45	0	10	0	10	311	2	
		Annex VII	26	21	0	6	0	1	47	7	
	Phase In	Annex X	142	6 244	0	1 694	43	875	772	390	
		Annex IX	124	734	0	788	24	385	1 171	93	
		Annex VIII	27	194	0	172	21	91	302	12	
		Annex VII	9	235	0	109	6	19	86	14	
Total			**375**	**7 608**	**0**	**2 941**	**94**	**1 451**	**2 887**	**535**	**15 891**
Acute toxicity (dermal)	Non-phase In	Annex X	12	147	0	78	0	31	23	8	
		Annex IX	31	120	0	66	0	15	26	1	
		Annex VIII	75	262	0	41	0	6	47	10	
		Annex VII	58	168	0	36	0	3	11	11	
	Phase In	Annex X	156	6 777	0	1 187	30	334	494	246	
		Annex IX	361	878	0	1 015	17	426	616	24	
		Annex VIII	67	203	0	228	12	146	148	12	
		Annex VII	28	187	0	45	1	33	67	7	
Total			**788**	**8 742**	**0**	**2 696**	**60**	**994**	**1 432**	**319**	**15 031**

Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Skin irritation/ corrosion	Non-phase In	Annex X	40	263	0	184	0	66	22	7	
		Annex IX	50	151	0	95	0	28	18	7	
		Annex VIII	122	323	0	54	3	15	49	12	
		Annex VII	402	458	0	93	1	24	43	10	
	Phase In	Annex X	351	14 208	0	2 097	43	1 128	242	410	
		Annex IX	639	2 050	0	1 593	52	753	316	238	
		Annex VIII	138	492	0	653	21	263	60	18	
		Annex VII	143	613	0	407	50	96	52	12	
Total			**1 885**	**18 558**	**0**	**5 176**	**170**	**2 373**	**802**	**714**	**29 678**
Eye irritation	Non-phase In	Annex X	37	152	0	134	0	53	22	7	
		Annex IX	44	136	0	83	0	15	20	4	
		Annex VIII	125	301	0	55	1	9	40	11	
		Annex VII	350	417	0	92	0	43	67	10	
	Phase In	Annex X	360	7 616	0	1 722	41	911	257	396	
		Annex IX	659	1 706	0	1 452	36	580	324	232	
		Annex VIII	130	451	0	601	21	164	63	23	
		Annex VII	130	538	0	280	30	67	63	9	
Total			**1 835**	**11 317**	**0**	**4 419**	**129**	**1 842**	**856**	**692**	**21 090**
Skin sensitisation	Non-phase In	Annex X	27	147	0	81	1	53	19	11	
		Annex IX	29	145	0	89	2	27	10	9	
		Annex VIII	94	313	0	47	2	22	9	12	
		Annex VII	312	433	0	93	0	33	42	12	
	Phase In	Annex X	264	6 345	0	1 486	69	1 148	301	522	
		Annex IX	519	1 120	0	1 364	51	887	251	199	
		Annex VIII	87	304	0	474	33	268	60	22	
		Annex VII	141	281	0	218	39	120	92	6	
Total			**1 473**	**9 088**	**0**	**3 852**	**197**	**2 558**	**784**	**793**	**18 745**

Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Repeated dose toxicity (oral)	Non-phase In	Annex X	16	63	2	109	0	57	51	1	
		Annex IX	66	177	19	122	1	25	62	8	
		Annex VIII	138	349	1	78	0	24	20	11	
		Annex VII	69	179	0	24	0	1	5	10	
	Phase In	Annex X	317	3 227	85	2 853	42	893	814	239	
		Annex IX	581	1 205	179	2 943	17	1 475	717	79	
		Annex VIII	147	280	12	690	17	357	74	17	
		Annex VII	41	259	6	290	4	30	34	14	
Total			**1 375**	**5 739**	**304**	**7 109**	**81**	**2 862**	**1 777**	**379**	**19 626**
Repeated dose toxicity (inhalation)	Non-phase In	Annex X	12	42	0	126	0	26	71	4	
		Annex IX	10	21	0	72	0	1	124	0	
		Annex VIII	8	11	1	5	0	1	144	1	
		Annex VII	7	0	0	3	0	0	12	0	
	Phase In	Annex X	168	3 715	7	2 004	43	264	1 056	246	
		Annex IX	91	412	22	761	24	179	976	19	
		Annex VIII	32	101	4	179	23	67	208	3	
		Annex VII	7	84	0	41	2	13	50	5	
Total			**335**	**4 386**	**34**	**3 191**	**92**	**551**	**2 641**	**278**	**11 508**
Repeated dose toxicity (dermal)	Non-phase In	Annex X	3	83	1	28	0	11	81	9	
		Annex IX	2	2	0	15	0	2	125	2	
		Annex VIII	4	22	0	10	0	2	136	0	
		Annex VII	2	4	0	1	0	1	11	0	
	Phase In	Annex X	8	5 860	3	446	0	64	1 355	165	
		Annex IX	12	228	1	250	1	57	1 090	5	
		Annex VIII	2	46	0	41	1	31	240	1	
		Annex VII	1	25	0	47	1	2	57	2	
Total			**34**	**6 270**	**5**	**838**	**3**	**170**	**3 095**	**184**	**10 599**

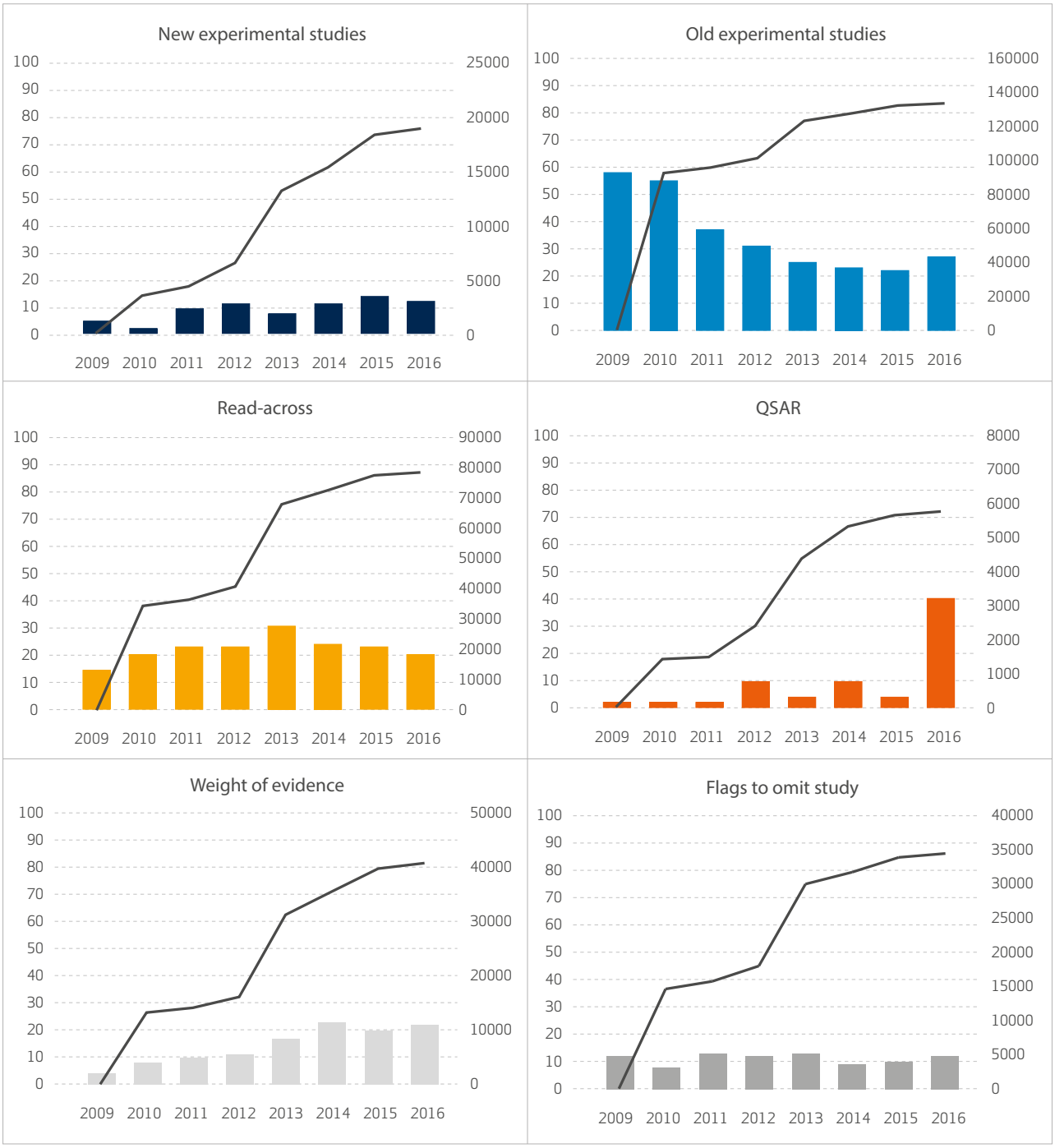
Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Genetic toxicity *in vitro*	Non-phase In	Annex X	65	154	0	197	0	86	16	8	
		Annex IX	80	146	0	159	1	50	17	6	
		Annex VIII	255	424	0	157	3	36	53	10	
		Annex VII	426	576	0	142	3	33	17	6	
	Phase In	Annex X	888	9 178	0	4 142	73	1 962	366	1 247	
		Annex IX	1 327	2 554	0	3 701	87	2 674	316	342	
		Annex VIII	356	852	0	1 358	35	828	54	28	
		Annex VII	195	975	0	549	77	290	44	28	
Total			**3 592**	**14 859**	**0**	**10 405**	**279**	**5 959**	**883**	**1 675**	**37 652**
Genetic toxicity *in vivo*	Non-phase In	Annex X	11	56	1	81	0	25	11	6	
		Annex IX	22	61	1	48	0	20	16	5	
		Annex VIII	55	139	4	31	0	16	6	9	
		Annex VII	35	99	1	14	1	0	0	12	
	Phase In	Annex X	153	3 420	10	2 040	1	573	229	340	
		Annex IX	118	683	30	995	21	603	134	28	
		Annex VIII	36	202	6	323	12	130	15	3	
		Annex VII	16	157	7	89	1	61	10	13	
Total			**446**	**4 817**	**60**	**3 621**	**36**	**1 428**	**421**	**416**	**11 245**
Carcinogenicity	Non-phase In	Annex X	0	23	0	77	0	25	52	12	
		Annex IX	2	11	0	41	0	4	20	5	
		Annex VIII	6	12	0	7	0	2	8	1	
		Annex VII	0	2	0	0	0	0	3	0	
	Phase In	Annex X	64	2 756	0	1 794	50	589	722	763	
		Annex IX	28	406	0	677	15	215	228	33	
		Annex VIII	16	154	0	183	24	47	25	17	
		Annex VII	2	182	0	114	1	10	19	11	
Total			**118**	**3 546**	**0**	**2 893**	**90**	**892**	**1 077**	**842**	**9 458**

Endpoint	Phase In Status	Annex	NES	OES	TP	RA	QS	WE	FO	MS	Total
Reproductive toxicity	Non-phase In	Annex X	18	26	7	96	0	75	64	4	
		Annex IX	68	28	4	76	0	27	137	6	
		Annex VIII	175	43	0	78	3	11	125	2	
		Annex VII	11	12	1	8	0	0	12	2	
	Phase In	Annex X	306	1 557	195	1 724	46	848	1 344	145	
		Annex IX	404	327	38	1 361	49	851	1 525	33	
		Annex VIII	117	88	3	301	20	266	209	13	
		Annex VII	28	56	3	103	4	46	78	4	
Total			**1 127**	**2 137**	**251**	**3 747**	**122**	**2 124**	**3 494**	**209**	**13 211**
Developmental toxicity/ teratogenicity	Non-phase In	Annex X	19	40	5	87	1	51	53	1	
		Annex IX	61	33	24	74	0	28	72	4	
		Annex VIII	85	41	1	75	0	6	25	0	
		Annex VII	13	17	0	4	0	0	3	1	
	Phase In	Annex X	317	3 342	129	2 460	42	907	1 045	204	
		Annex IX	260	634	275	1 950	29	1 019	789	38	
		Annex VIII	53	120	18	368	16	270	127	12	
		Annex VII	20	107	8	220	13	11	61	5	
Total			**828**	**4 334**	**460**	**5 238**	**101**	**2 292**	**2 175**	**265**	**15 693**

Appendix 3. Trend analysis

Figure 3.1 illustrates what options registrants chose in their initial dossier by year during the study period. This temporal analysis is shown for the first time in this report.

Figure 3.1. Total number of endpoint study records submitted by registrants per option and year, normalised by total number of endpoint study records received each year (the bars) and cumulative number of endpoint study records per year (the line).



The values of the bars are normalised values according to the total number of ESRs per year. The values for the line are not normalised (cumulative). This means that for each year, the number of ESRs received in this year and all the previous years is counted. It is noted that 2016 is not truly representative for the trends since the data for analysis is taken at the end of March 2016.

What can be seen from the graphs is that the proportion of use of old experimental studies declines over the years while the use of weight of evidence slightly increases. The overall trends of the relative use of the different options to address information requirements seen in the trend analysis are almost uniform across the endpoints.

In total numbers, the most common adaptation seems to be read-across, (with a total number of ESRs ca. 80 000), followed by weight of evidence (ca. 40 000), followed by data waiving (ca. 34 000). QSAR is present only in ca. 6 000 records. The cumulative number of testing proposals amount just to ca. 1 800. Due to the relatively low number of testing proposals compared to other options, the graph on testing proposals has not been included in these results and have been discussed separately in Section 3.2.5 of the report.

Appendix 4: Analysis of adaptations to the standard information requirements

The total number of ESRs with read-across identified according the criteria described above is 80 722.

The number of ESRs per endpoint and per purpose flag are shown in Table 4.1.

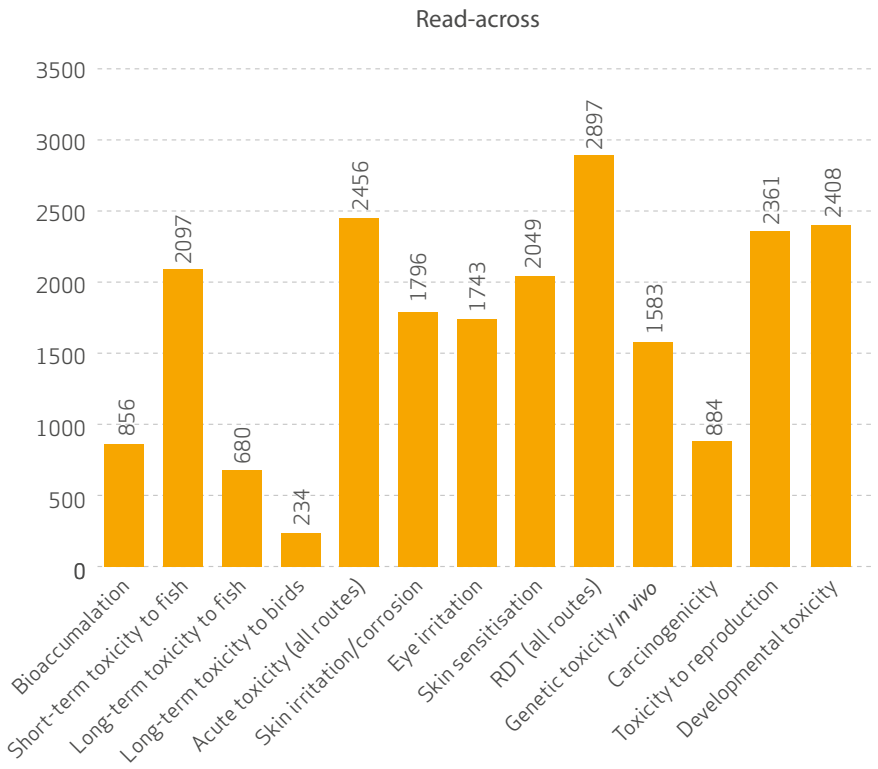
Notably, one substance may contain more than one ESR with read-across for a given endpoint. The number of substances per endpoint containing at least one read-across ESR is shown in Figure 4.1. The total number of substances with at least one read-across ESR per endpoint is 3 941.

Generally, from Table 4.1 it can be seen that the read-across is used as a key study more often for high tier endpoints, e.g. toxicity to reproduction, developmental toxicity and long-term toxicity to fish. The difference is also visible for skin sensitisation, which is not traditionally considered as high tier endpoint. For low tier endpoints, like skin irritation/corrosion, eye irritation, acute toxicity (all routes), and the use of read-across as a key and as a supporting study is almost equal.

Table 4.1: Distribution of ESRs with read-across per purpose flag and per endpoint

ESRs WITH READ-ACROSS					
Endpoint name	Key Study	Supporting Study	Weight of Evidence	Not Defined	Total
Bioaccumulation	742	601	931	130	2 404
Short-term toxicity to fish	2 542	3 783	2 409	545	9 279
Long-term toxicity to fish	1 577	1 277	423	240	3 517
Long-term toxicity to birds	305	347	420	17	1 089
Acute toxicity (all routes)	5 440	5 460	2 422	430	13 752
Skin irritation/corrosion	2 397	2 607	1 056	183	6 243
Eye irritation	2 087	2 141	756	197	5 181
Skin sensitisation	2 295	1 481	1 338	83	5 197
Repeated dose toxicity (all routes)	4 879	5 962	2 623	347	13 811
Genetic toxicity *in vivo*	2 151	1 340	772	139	4 402
Carcinogenicity	1 407	1 403	456	83	3 349
Toxicity to reproduction	2 593	1 093	1 617	130	5 433
Developmental toxicity	2 888	2 195	1 762	220	7 065
Total	**31 303**	**29 690**	**16 985**	**2 744**	**80 722**

Figure 4.1: Number of substances with read-across by endpoint



4.1 THE USE OF QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) APPROACHES

ESRs with IUCLID study result type "(Q)SAR" and "estimated by calculation" (as specified by the registrant) were counted both as "QSAR" for this analysis.

The total numbers of ESRs for QSAR are shown in Table 4.2. The total number of ESRs using "calculated results", counted as described above, is 7 822.

The transformation of this number into number of substances, defined by EC number and containing at least one QSAR resulted in a total of 2 135 substances. The total number of substances containing at least one QSAR is 2 135, the number of QSARs per substances for each endpoint is shown in Figure 4.2.

The disproportional increase of QSAR for bioaccumulation between this and the previous report compared to other endpoints can be explained by the adapted methodology.

While in the previous report only ESRs were counted that specifically referred to fish, the methodology for the current report was adapted to exclude any ESR referring to invertebrates. For this exclusion, the information on species, but also the title of the study report or information about QSAR models were taken into account. For example, any QSAR prediction based on the US EPA BCFBAF model was considered to be related to vertebrate animals in this report.

Table 4.2: Number of ESRs with QSAR and "calculated results" per purpose flag and per endpoint

ESRs WITH QSAR AND CALCULATED RESULT

Endpoint name	Key Study	Supporting Study	Wight of Evidence	Not Defined	Total
Bioaccumulation	1 288	1 103	1 606	79	4 076
Short-term toxicity to fish	380	534	254	28	1 196
Long-term toxicity to fish	327	125	97	11	560
Long-term toxicity to birds	3	0	0	0	3
Acute toxicity (all routes)	236	48	116	9	409
Skin irritation/corrosion	110	60	66	0	236
Eye irritation	90	39	46	0	175
Skin sensitisation	131	65	186	1	383
Repeated dose toxicity (all routes)	148	26	75	2	251
Genetic toxicity *in vivo*	19	17	10	0	46
Carcinogenicity	78	9	17	3	107
Toxicity to reproduction	94	27	89	1	211
Developmental toxicity	76	24	68	1	169
Total	**2 980**	**2 077**	**2 630**	**135**	**7 822**

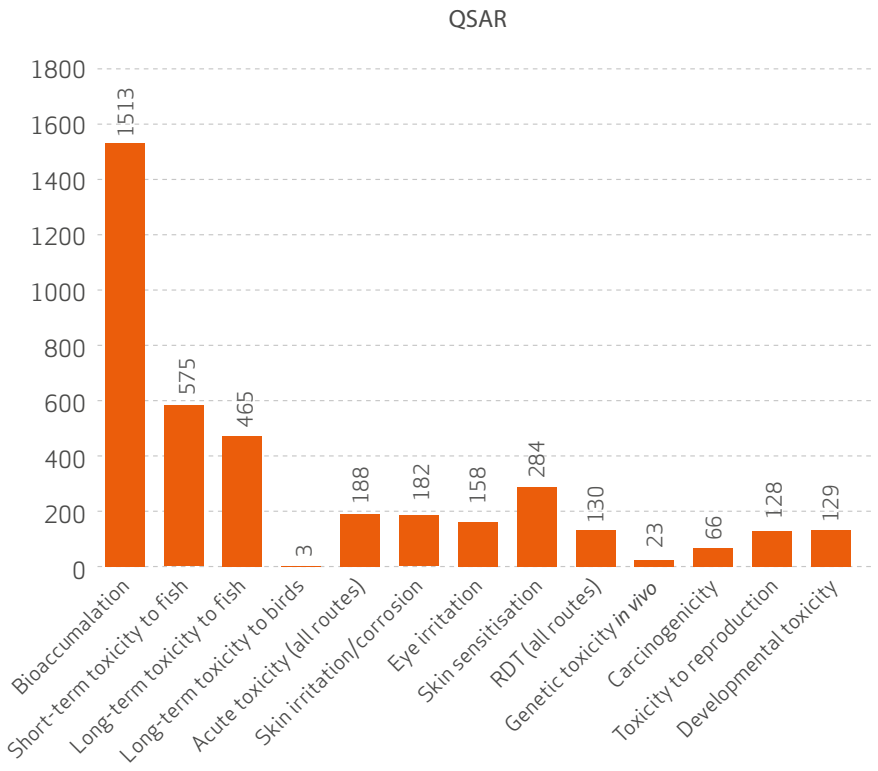
As can be seen from Table 4.2, QSAR was mostly flagged as a key study rather than as a supporting study. An exception is the endpoint "short-term toxicity to fish", where the large amount of experimental data and enhanced possibilities for read-across are probably preferred to QSARs.

Nevertheless, QSARs are also used for this endpoint and in principle can predict the toxicity in a relatively reliable manner if the substance is within the applicability domain of the QSAR model. The use of QSAR for bioaccumulation as a supporting study is also high.

The pattern of the distribution on substance level is similar to the 2014 report but the use of QSAR for bioaccumulation increases more than for the other aquatic endpoints. The explanation is a refined methodology as explained above.

QSAR is used occasionally for human health endpoints. Presumably, both expert systems and statistical methods were used. QSAR were used most for predicting skin sensitisation. The use of QSAR for other health endpoints is lower.

Figure 4.2: Number of substances with QSAR and "calculated results" by endpoint



4.2 THE USE OF WEIGHT OF EVIDENCE APPROACHES

ESRs with the IUCLID purpose flag "Weight of Evidence" as specified by the registrant have been counted as follows:

- ESRs with study result type: "Experimental result" were counted as old experimental studies (OES), and new experimental studies (NES), depending on the report date of the study;
- ESRs with study result type: "Read-across based on grouping of substances" and "read-across from supporting substance" were counted as read-across (RA);
- ESRs with study result type: "(Q)SAR" and "estimated by calculation" were counted as QSARs (QS);
- Data waiving was counted as flags to omit study (FO);
- There were also other study types, which are denoted as miscellaneous (MS).

The ESRs with weight of evidence per endpoint and per study result type are shown in Table 4.3. There are a total of 31 325 ESRs counted. These ESRs translated to a total of 2 708 substances. The number of QSARs per substances for each endpoint is shown in Figure 4.3.

The weight of evidence seems to be based mainly on old experimental studies and read-across. New experimental studies are considerably used less in weight of evidence. QSARs were used less than read-across. It can be seen that the weight of evidence is used more for high tier human health endpoints.

Table 4.3: Distribution of ESRs with weight of evidence per study result type and per endpoint. Legend: OES – old experimental studies; NES – new experimental studies; WE – weight of evidence; RA – read-across; QS – QSAR; TP – testing proposal; FO – flags to omit study; MS – miscellaneous

ESRs WITH WEIGHT OF EVIDENCE

Endpoint name	NES	OES	RA	QS	FO	MS	Total
Bioaccumulation	56	1 003	931	1 606	28	374	3 998
Short-term toxicity to fish	79	596	2 409	254	27	111	3 476
Long-term toxicity to fish	108	414	423	97	27	130	1 199
Long-term toxicity to birds	0	60	420	0	25	555	1 060
Acute toxicity (all routes)	43	1 704	2 422	116	74	141	4 500
Skin irritation/corrosion	125	1 087	1 056	66	19	20	2 373
Eye irritation	108	886	756	46	27	19	1 842
Skin sensitisation	125	861	1 338	186	23	25	2 558
Repeated dose toxicity (all routes)	59	727	2 623	75	52	47	3 583
Genetic toxicity *in vivo*	187	434	772	10	0	25	1 428
Carcinogenicity	5	290	456	17	0	124	892
Toxicity to reproduction	36	335	1 617	89	33	14	2 124
Developmental toxicity	25	388	1 762	68	29	20	2 292
Total	**956**	**8 785**	**16 985**	**2 630**	**364**	**1 605**	**31 325**

Figure 4.3: Distribution of substances containing at least one weight of evidence as a study result type

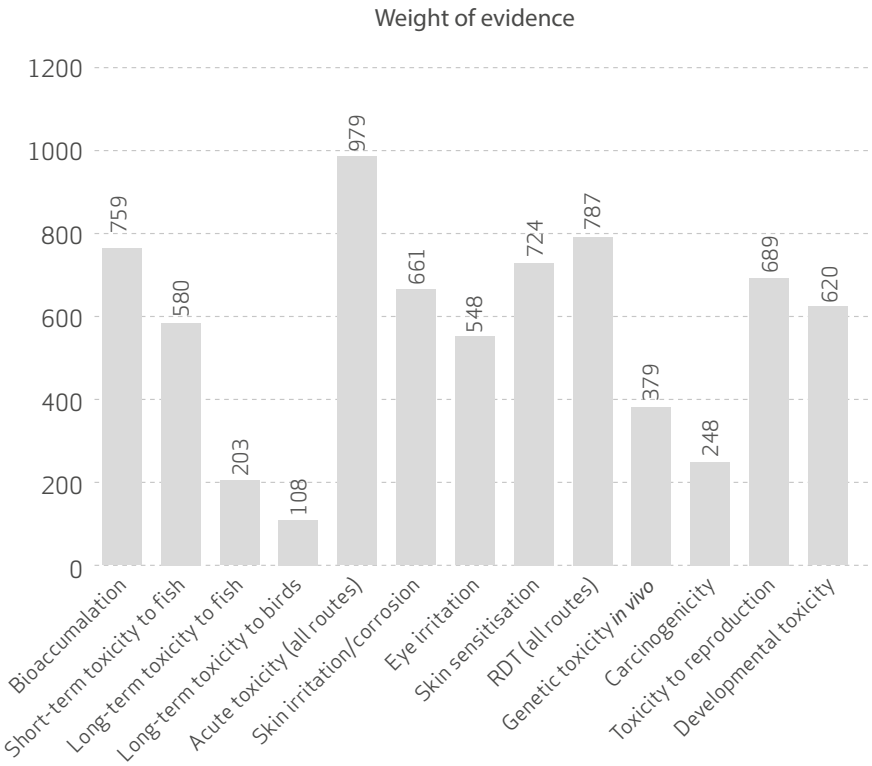
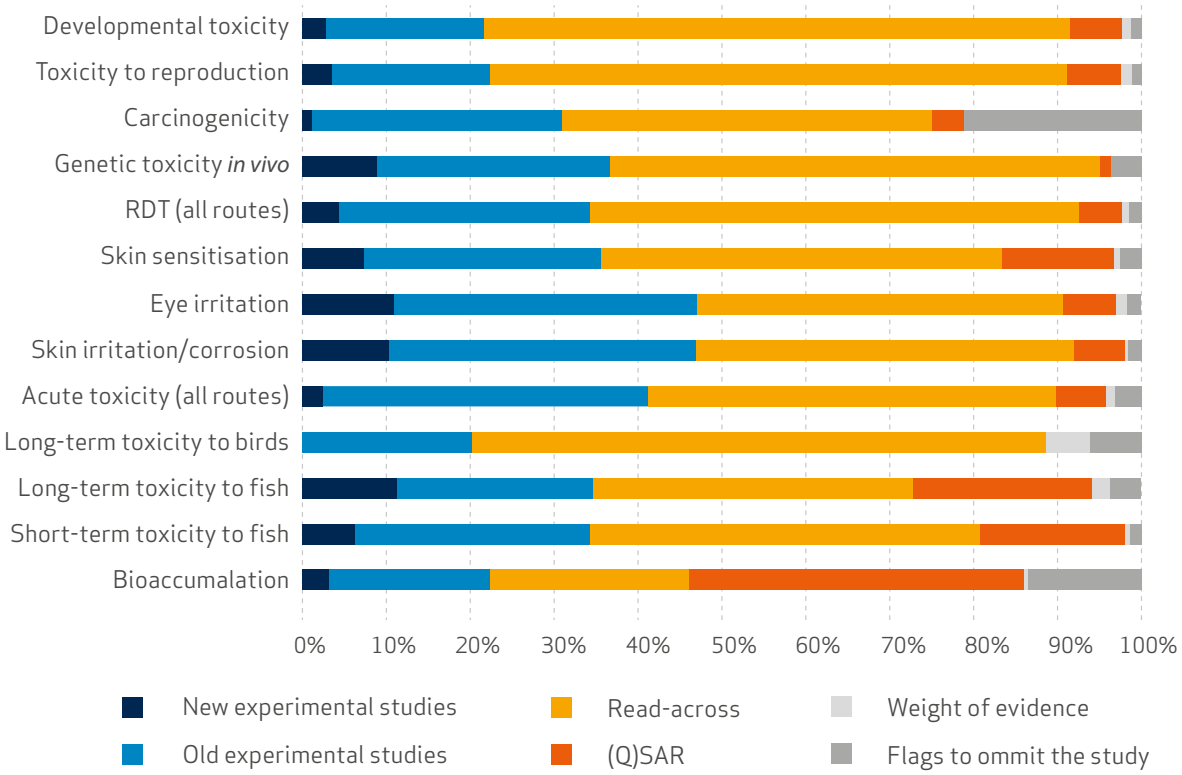


Figure 4.4: Distribution of substances with a weight of evidence flag



As can be seen in Figure 4.4, weight of evidence was used most frequently for the endpoints acute toxicity, all routes (979 substances) and repeated dose toxicity, all routes (787 substances). Weight of evidence was found for bioaccumulation (759 substances) and skin sensitisation (724 substances). Less frequently, weight of evidence was used for carcinogenicity to birds (108 substances) and long-term toxicity to fish (203) substances.

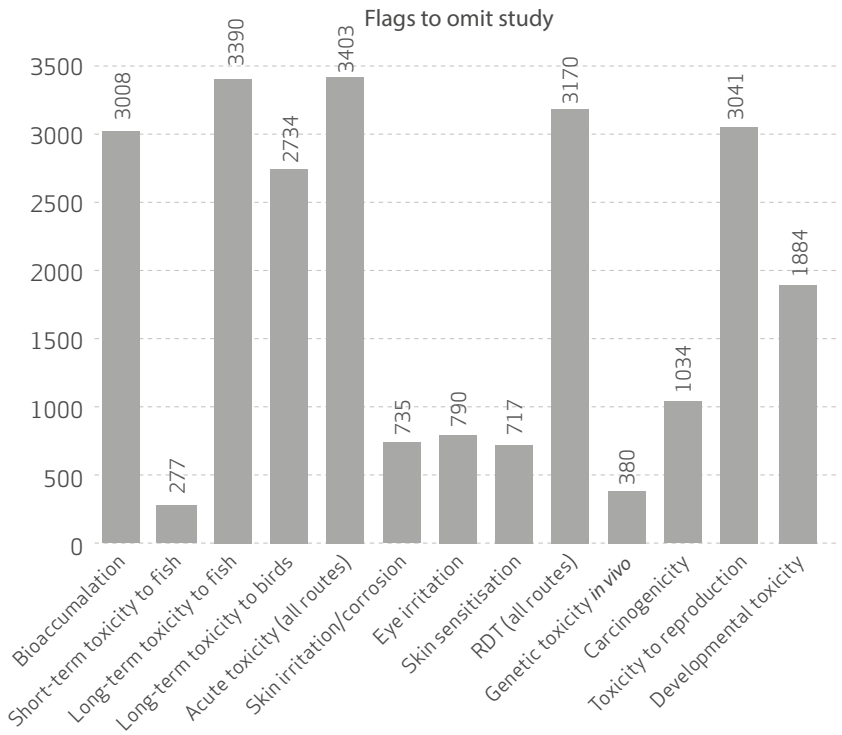
4.3 DATA WAIVING

Registrants used opportunities, which REACH gives to waive studies, either by specific rules in Column 2 of the REACH Annexes VII-X, or by the adaptation possibility provided in the REACH Annex XI. Waiving was not addressed in detail in the previous reports but is included in this report because a considerable number of waiving arguments have been seen: at least one waiving argument was found in 5 353 substances.

Looking at the distribution of ESRs (Table 4.4), it is possible to see that the highest number of individual waiving arguments was found in the endpoint "toxicity to reproduction". Many ESRs referring to data waiving were found in the inhalation and dermal routes of repeated dose toxicity and in the inhalation route of acute toxicity.

The interpretation of data waiving is not straightforward. Since some studies are conditional, e.g. genetic toxicity *in vivo*, not many waiving arguments were presented since animal testing depends on an integrated strategy. A similar conclusion could be derived for carcinogenicity.

Figure 4.5: Distribution of substances with waiving arguments per endpoint



For the low tier human health endpoints, less data waiving is done compared to the high tier tests. For these endpoints, the informational requirements were met either by experimental data (including *in vitro* tests), or by other adaptations such as read-across, weight of evidence and QSAR, as has been shown in previous projections.

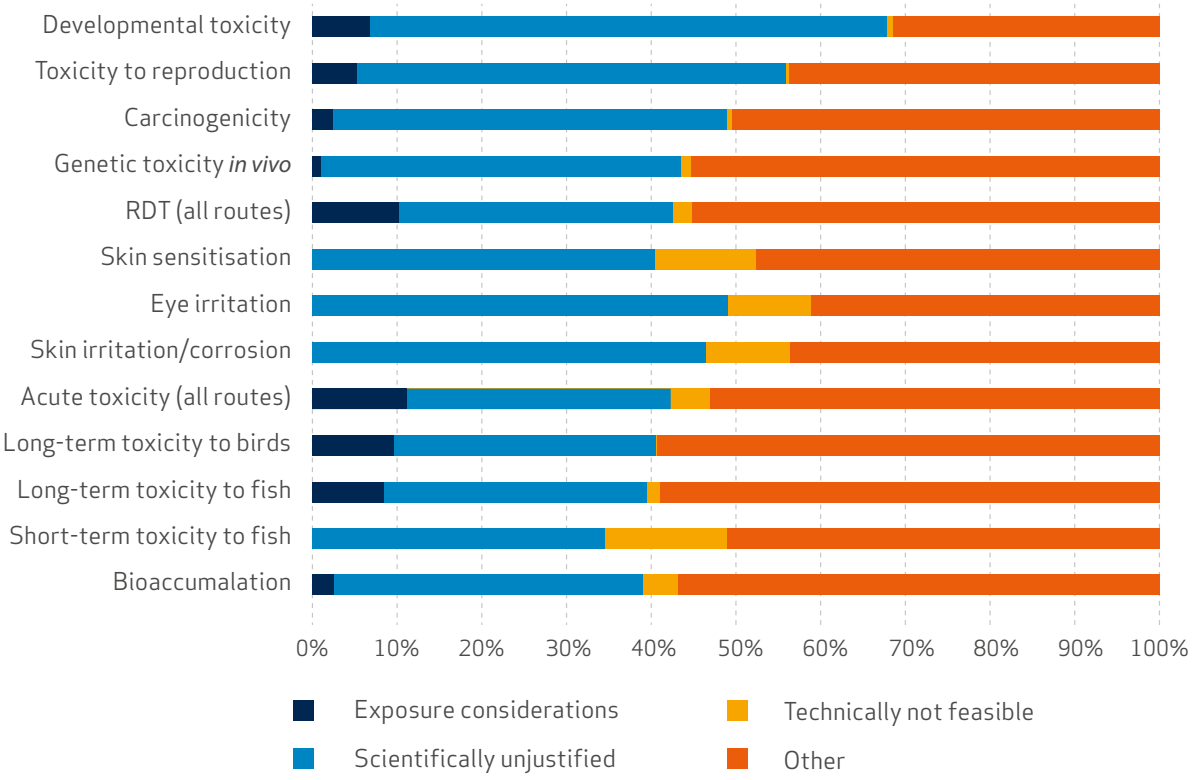
Table 4.4: Distribution of ESRs with waiving arguments depending on the legal basis for data waving

ESRs WITH FLAGS FOR OMITTING STUDIES

Endpoint name	Exposure considerations	Scientifically unjustified	Technically not feasible	Other	Total
Bioaccumulation	82	1 207	137	1 882	3 308
Short-term toxicity to fish	0	115	48	170	333
Long-term toxicity to fish	306	1 136	55	2 157	3 654
Long-term toxicity to birds	286	926	5	1 775	2 992
Acute toxicity (all routes)	527	1 480	219	2 515	4 741
Skin irritation/corrosion	0	374	81	351	806
Eye irritation	0	429	86	360	875
Skin sensitisation	0	322	95	381	798
Repeated dose toxicity (all routes)	770	2 445	167	4 166	7 548
Genetic toxicity *in vivo*	4	176	5	229	414
Carcinogenicity	26	501	7	543	1 077
Toxicity to reproduction	183	1 785	13	1 540	3 521
Developmental toxicity	148	1 335	15	690	2 188
Total	**2 332**	**12 231**	**933**	**16 759**	**32 255**

Figure 4.6 shows the distribution of data waiving arguments by legal basis (i.e. exposure considerations, scientifically unjustified, technically not feasible and other). The total number of ESRs with data-waiving arguments sum up to 100 %.

Figure 4.6: Distribution of data waiving arguments used in registration dossiers.

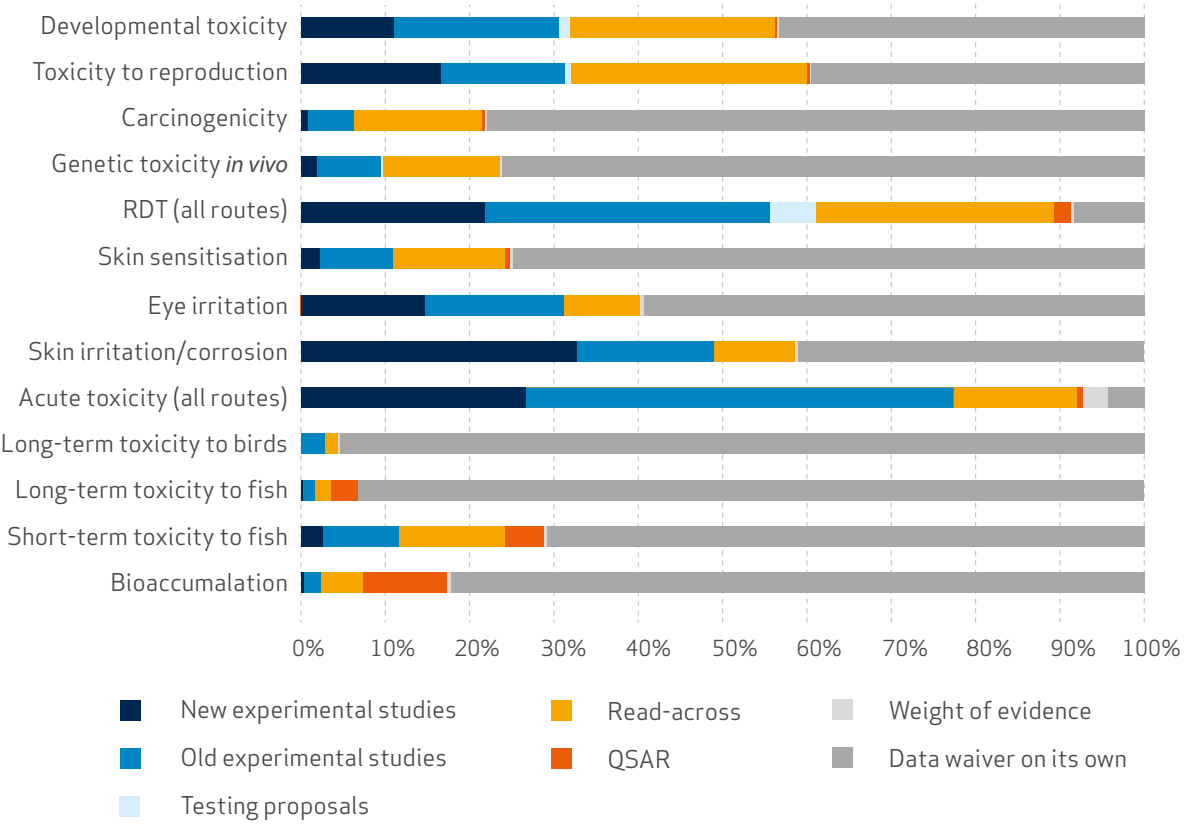


One important observation is that many registrants preferred "other" to report their data waiving justification. A more detailed analysis of the justifications where "other" was selected indicates that in the majority of cases (approx. 55 %) the argumentation was based on Column 2 endpoint specific adaptations. Not relevant route of exposure was waived using "other" in approx. 12 % of cases. Approximately 10 % of "other" data-waiving arguments were related to exposure considerations. The remaining cases can be mainly characterised as testing scientifically unjustified.

In general, for more than half of the cases, data waiving was not combined with other options to cover information requirement (53 %). In 28 % of cases registrants combined them with experimental data (11 % with new and 17 % with old experimental studies).

In addition, in 14 % of cases data waiving arguments were combined with read-across. Figure 4.7 presents the distribution over the analysed endpoints. Data-waiving arguments are mainly combined with other options (acute and repeated dose toxicity) where not relevant routes of exposure can be waived. Also, for endpoints like toxicity to reproduction, developmental toxicity and skin irritation corrosion, data-waiving arguments were mainly combined with other options. For the remaining endpoints, data waiving arguments are mainly used as standalone.

Figure 4.7: Distribution of data waiving on its own and in combination with other options.



4.4 AGGREGATION OF ENDPOINTS PER SUBSTANCE

The aggregated number of substances with adaptation per endpoint are shown in Table 4.5. Since there were often several adaptations found for the same endpoint in one substance, the number of unique substances affected (as unique EC numbers) was counted separately per adaptation (and is not deducible in previous tables).

The number of substances per endpoint is a total number of substances, which contain any information for the endpoint. This is not necessarily information from standard OECD/EU guideline tests. This total number was found useful when analysing how adaptations were used because it provided a denominator for normalisation of the number of substances with a given adaptation per endpoint.

Counting the substances with at least one adaptation, clearly waving was used most often (ca. 5 300), followed by read-across (ca. 3 900), then followed by weight of evidence (ca. 2 700), and then by QSAR (2 135) substances.

In terms of increase from the previous report (in ESRs), the largest step of increase is seen in QSARs (2.7 times), compared to weight of evidence (1.7 times), and read-across (1.4 times). Flags to omit studies cannot be compared because these were not analysed in the previous report.

Table 4.5: Distribution of substances per adaptation and per endpoint

SUBSTANCES WITH ADAPTATIONS PER ENDPOINT

Endpoint name	RA	QS	WE	FO	Endpoint
Bioaccumulation	865	1 513	759	3 008	4 928
Short-term toxicity to fish	2 097	575	580	277	5 591
Long-term toxicity to fish	680	465	203	3 390	4 723
Long-term toxicity to birds	234	3	108	2 734	3 040
Acute toxicity (all routes)	2 456	188	979	3 403	6 236
Skin irritation/corrosion	1 796	182	661	735	6 237
Eye irritation	1 743	158	548	790	6 233
Skin sensitisation	2 049	284	724	717	6 231
Repeated dose toxicity (all routes)	2 897	130	787	3 170	5 437
Genetic toxicity *in vivo*	1 583	23	379	380	3 477
Carcinogenicity	884	66	248	1 034	2 507
Toxicity to reproduction	2 361	128	689	3 041	5 207
Developmental toxicity	2 408	129	620	1 884	4 939

Appendix 5. Skin corrosion/irritation

The studies used to investigate corrosion and/or irritation predict the local effects of the test substance on humans at the site of first contact (skin, eye, or the mucous membrane of respiratory or gastrointestinal tract) after a single exposure. Observed local effects can be further differentiated as either corrosive or irritant effects, depending on their severity, reversibility or irreversibility.

There are *in vitro* methods available that are scientifically valid and have internationally adopted test guidelines for this endpoint. Those methods should be used by registrants to fulfil the information requirements for this endpoint either as a standalone method depending on the outcome or in a tiered testing strategy 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, EU B.40/OECD TG 430) test, the human skin model test (based on reconstructed human epidermis, EU B.40bis/ OECD TG 431) and the membrane barrier test (OECD TG 435).

Positive results from such methods are sufficient to conclude that the substance is corrosive to the skin and no further testing *in vitro* or *in vivo* is needed. For negative outcomes i.e. non-corrosive, an *in vitro* skin irritation test is needed to assess whether the substance causes skin irritation or not. For skin irritation, reconstructed human epidermis (EU B.46/OECD TG 439) test methods are available.

A negative result from such tests is sufficient to conclude that no classification is needed and no further testing *in vitro* or *in vivo* is needed. For a positive outcome, an *in vitro* skin corrosion test is needed to assess whether the substance is irritant or corrosive to the skin.

For *in vivo* studies conducted according to EU B.4/OECD TG 404, 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 effects of the substance on the animals are usually monitored for 72 hours and even up to 14 days and reported in a standardised format.

The standard information requirements for this endpoint were amended on 31 May 2016 and entered into force on 20 June 2016, where the standard information on an *in vivo* study at REACH Annex VIII level was removed.

The information requirements are provided in Annexes VII and VIII to REACH and differ depending on the tonnage band. Annex VII (1 to 10 tpa) requires only *in vitro* studies, while at REACH Annex VIII-X level and above (more than 10 tpa) requires an *in vivo* test to be performed only if the *in vitro* studies listed under REACH Annex VII are not applicable, or the results of these studies are not adequate for classification and risk assessment.

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 to REACH. The potential to cause irritation or corrosion can also be predicted based on physico-chemical properties of the chemical (for example, the substance is a strong acid/base).

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 made use of alternative test methods to fulfil the standard information requirements for their substance.

For this substance level analysis, 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 3.1 of this report, is due to the fact that the *in vitro* skin irritation test methods were only adopted in July 2009 by the European Test Methods Regulation. Therefore, it was decided to have a cut off year of 2010 to allow registrants to take these new developments into account in their testing strategy.

In total, 5 451 *in vitro* endpoint study records (ESRs), containing information either from the registered substance or from the read-across substance, were submitted by the end of March 2016 (by the 2013 registration deadline, 1 184 *in vitro* ESRs were submitted).

The total number of *in vitro* ESRs provided by the end of March 2016 has increased substantially, which is mainly because all tonnage bands are covered in this report i.e. more substances are evaluated, as in the previous report only substances registered at 100 to more than 1 000 tpa were evaluated.

For the two highest tonnage bands, the number of submitted *in vitro* ESRs (for the registered substance or read-across substance) has increased more than 2.5-fold (4 770 *in vitro* ESRs submitted) when compared to the number submitted by the 2013 registration deadline (1 184 *in vitro* ESRs). This shows that registrants are using *in vitro* approaches by either using information from the registered substance or from the read-across substance more than they were in the data analysed in 2014.

For 702 dossiers, the information requirement was fulfilled solely by using *in vitro* methods (10.6 % of total dossier submissions). For 627 dossiers (9.5 %), the *in vitro* methods were performed with the registered substance and for 75 dossiers (1.1 %) *in vitro* methods were performed with a read-across substance.

In vitro methods were also extensively used together with old *in vivo* studies (715 dossiers and 10.8 % of total dossier submissions). For 649 dossiers (9.8 %), at least one *in vitro* method was performed with the registered substance and *in vivo* methods were performed either with the registered or read-across substance. For 66 dossiers (1.0%), *in vitro* methods were performed with a read-across substance and *in vivo* methods were performed either with registered or read-across substances.

This shows that registrants are relying on *in vitro* methods and are using those methods to provide support when only old *in vivo* data is available and when a read-across approach is followed or by building a read-across adaptation solely based on those.

New (2009 or later) *in vivo* experimental tests have been performed for skin corrosion/irritation across all tonnage bands assessed. Where information on a new *in vivo* test has been submitted without prior performance of an *in vitro* test, ECHA analysed a sample of cases to investigate the reasons behind such behaviour. The initial findings of a sample of cases suggests that, in most cases, studies were performed for other regulatory purposes.

The registrants continue using read-across approaches (category or analogue) and 988 dossiers (14.9 % of total dossier submissions) contained *in vitro* or *in vivo* information solely on a read-across substance. From the 988 dossiers, 799 of them contained solely *in vivo* read-across data (12.1 % of 6 622 dossiers analysed), 122 dossiers contained solely *in vitro* and *in vivo* read-across data (1.9 % of the 6 622 dossiers analysed) and 67 dossiers contained solely *in vitro* read-across data (1.0 % of the 6 622 dossiers analysed). It is to 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.

Registrants are still making use of QSARs and calculations to a limited extent. In the majority of cases, registrants have used QSARs 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 QSAR estimations. ECHA notes that QSAR estimations could be more helpful when identifying substances requiring classification, but are less useful to provide alerts on non-irritant substances.

Table 5.1: Type of dossiers submitted for skin corrosion/irritation endpoint for all annexes (6 622 substances analysed)[a]

	Number of dossiers	% of total dossiers number	Dossier and ESR ratio[b]
Dossiers with only *in vitro*[c]	702	10.6	1:1.5
Dossiers with only in vitro for registered substance	*627*	*9.5*	*1:1.5*
Dossier with only in vitro read-across data	*75*	*1.1*	*1:2*
Dossiers with *in vitro* and *in vivo*[d]	715	10.8	1:6 *in vitro* 1:10 *in vivo*
Dossiers with in vitro and old in vivo[e]	*274*	*4.1*	*1:1.5 in vitro* *1:3 in vivo*
Dossiers with in vitro and old read-across in vivo[f]	*226*	*3.4*	*1:16 in vitro* *1:25 in vivo*
Dossiers with in vitro and new in vivo[e]	*165*	*2.5*	*1:1.5 in vitro* *1:1 in vivo*
Dossiers with in vitro and new read-across in vivo[g]	*50*	*0.8*	*1:2 in vitro* *1:10 in vivo*
Dossiers with old *in vivo*[e]	3 804	57.4	1:3
Dossiers with solely old *in vivo* read-across data[f]	775	11.7	1:3
Dossiers with new *in vivo* for the registered substance[e]	212	3.2	1:1
Dossiers with new *in vivo* read-across data[g]	66	1.0	1:2.5
Dossiers with only QSARs, or estimations by calculation	78	1.2	1:1
Dossiers with only waiving statements	270	4.1	1:1

a From the pool, duplicate dossiers for one substance were removed i.e. the number of dossiers analysed reflects the number of substances.

b These ratios are approximate values for illustrative purposes.

c The breakdown of use of in vitro methods reported below.

d The breakdown of use of in vitro and in vivo methods reported below.

e Substances contain at least one in vivo study performed with the registered substance, but may also contain studies performed with a read-across substance, QSARs and waiving statements.

f In the dossier, all in vivo studies have been performed with a read-across substance, but may also contain ESRs for QSARs and waiving statements.

g In the dossier, at least one new in vivo study has been performed with a read-across substance, but may also contain old read-across studies. They may also contain ESRs for QSARs and waiving statements.

Table 5.2: Breakdown of information provided per Annex (Annex VII - 1 266, Annex VIII - 767, Annex IX - 2 205 and Annex X - 2 384 substances assessed)[a]

	Annex VII number of dossiers	% of total dossier number	Annex VIII number of dossiers	% of total dossier number	Annex IX number of dossiers	% of total dossier number	Annex X number of dossiers	% of total dossier number
Dossiers with only *in vitro*[c]	272	21.6	80	10.4	248	11.3	100	4.2
Dossiers with only in vitro on registered substance	*260*	*20.5*	*72*	*9.4*	*210*	*9.6*	*85*	*3.6*
Dossiers with only in vitro on read-across substance	*14*	*1.1*	*8*	*1.0*	*38*	*1.7*	*15*	*0.6*
Dossiers with *in vitro* and *in vivo*[d]	68	5.4	79	10.3	291	13.2	277	11.6
Dossiers with in vitro and old in vivo	*40*	*3.2*	*25*	*3.3*	*98*	*4.4*	*111*	4.7
Dossiers with in vitro and old read-across in vivo	*9*	*0.7*	*20*	*2.6*	*79*	*3.6*	*118*	4.9
Dossiers with in vitro and new in vivo[e]	*18*	*1.4*	*28*	*3.6*	*80*	*3.6*	*39*	1.6
Dossiers with in vitro and new read-across in vivo[g]	*1*	*0.1*	*6*	*0.8*	*34*	*1.6*	*9*	0.4
Dossiers with old *in vivo*[e]	708	55.9	463	60.4	1 154	52.3	1 479	62.0
Dossiers with solely old *in vivo* read-across[f]	73	5.8	68	8.9	287	13.0	347	14.6
Dossiers with new *in vivo* for the registered substance[e]	57	4.5	39	5.1	88	4.0	28	1.2
Dossiers with new *in vivo* read-across[g]	18	1.4	9	1.2	29	1.3	10	0.4
Dossiers with only QSARs, or estimations by calculation	19	1.5	8	1.0	22	1.0	29	0.4
Dossiers with only waiving statements	49	3.9	21	2.7	86	3.9	114	4.8

a From the pool, duplicate dossiers for one substance were removed i.e. the number of dossiers analysed reflects the number of substances.

c The breakdown of use of *in vitro* methods reported below.

d The breakdown of use of *in vitro* and *in vivo* methods reported below.

e Substances contain at least one *in vivo* study performed with the registered substance, but may also contain studies performed with a read-across substance, QSARs and waiving statements.

f In the dossier, all *in vivo* studies have been performed with a read-across substance, but may also contain ESRs for QSARs and waiving statements.

g In the dossier, at least one new *in vivo* study has been performed with a read-across substance, but may also contain old read-across studies. They may also contain ESRs for QSARs and waiving statements.

Appendix 6. Serious eye damage/Eye irritation

As with the skin corrosion/irritation endpoint (see Appendix 5), 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.

There are *in vitro* methods that are scientifically valid and have internationally adopted test guidelines. Those methods should be used by registrants to fulfil the information requirements for this endpoint either as a standalone method depending on the outcome or in a tiered testing strategy to fully replace testing on animals.

A positive or a negative outcome from an *in vitro* assay such as the bovine corneal opacity and permeability (BCOP, EU B.47/OECD TG 437), isolated chicken eye (ICE, EU B.48/OECD TG 438) or short time exposure (STE, OECD TG 491) tests is sufficient to classify substances as inducing serious eye damage (Category 1) or as not requiring classification (no such Category) under REACH and CLP no further testing *in vitro* or *in vivo* is needed to fulfil the standard information requirements.

A positive outcome from an *in vitro* assay such as the fluorescein leakage (FL, OECD TG 460) test method is sufficient to classify substances as inducing serious eye damage (Category 1) and no further testing *in vitro* or *in vivo* is needed.

A negative outcome from an *in vitro* assay such as the reconstructed cornea-like epithelium (RhCE, OECD TG 492) is sufficient to conclude that no classification or further testing *in vitro* or *in vivo* is needed.

For *in vivo* studies conducted according to EU B.5/OECD TG 405, the substance to be tested is applied in a single dose to the eye of an experimental animal for 24 hours, usually an 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 standard information requirements for this endpoint were amended on 31 May 2016 and entered into force on 20 June 2016, where the standard information on the *in vivo* study at the REACH Annex VIII level was removed. The information requirements are provided in Annexes VII and VIII to REACH and differ depending on the tonnage band.

REACH Annex VII (1-10 tpa) requires only *in vitro* studies, while at the REACH Annex VIII-X level and above (more than 10 tpa), an *in vivo* test must only be performed if the *in vitro* studies listed under REACH Annex VII are not applicable, or the results of these studies are not adequate for classification and risk assessment.

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 to REACH. The potential to cause serious eye damage can also be predicted based on physico-chemical properties of the chemical (for example, the substance is a strong acid/base).

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 concentrated more on the substance level to obtain an understanding of how registrants have made use of alternative test methods to fulfil the standard information requirements for their substances. For this substance level analysis, a cut off year of 2010 was chosen to identify new *in vivo* studies. The reason for deviating from the cut off year of 2009 specified in Section 3.1 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, it was decided to place the cut off year at 2010 to allow registrants to take these new developments into account in their testing strategies.

In total, 1 641 *in vitro* endpoint study records (ESRs), containing information either from the registered substance or from a read-across substance, were submitted by the end of March 2016 (by the 2013 registration deadline, 834 *in vitro* ESRs were submitted).

The total number of *in vitro* ESRs provided by the end of March 2016 has increased by more than 100 %. This increase is mainly because all tonnage bands are covered in this report i.e. more substances are evaluated, as in the previous report only substances registered at 100 to more than 1 000 tpa were evaluated.

For the two highest tonnage bands, the number of submitted *in vitro* ESRs (for the registered substance or read-across substance) has increased by 25 % (1 046 *in vitro* ESRs submitted) when compared to the number submitted by the 2013 registration deadline (834 *in vitro* ESRs submitted). This shows that registrants are using *in vitro* approaches by either using information from the registered substance or from a read-across substance more than they were in the data analysed in 2014.

In 476 dossiers, the information requirement was fulfilled solely by using *in vitro* methods (7.2 % of the total dossier submissions). For 435 dossiers (6.6 %), the *in vitro* methods were performed with the registered substance and for 40 dossiers (0.6 %) *in vitro* methods were performed with a read-across substance.

In vitro methods were also extensively used together with old *in vivo* studies (803 dossiers and 12.1 % of total dossier submissions). For 668 dossiers (10.2 %), at least one *in vitro* method was performed with the registered substance and *in vivo* methods were performed either with the registered or read-across substance. For 135 dossiers (2.0 %), *in vitro* methods were performed with a read-across substance and *in vivo* methods were performed either with a registered or read-across substance.

This shows that the registrants are relying on *in vitro* methods and are using those methods to provide support when only old *in vivo* data is available and when a read-across approach is followed or by building a read-across adaptation solely based on those.

New (2009 or later) *in vivo* experimental tests have been performed for serious eye damage/eye irritation across all tonnage bands assessed. Where information on a new *in vivo* test has been submitted without prior performance of an *in vitro* test, ECHA analysed a sample of cases to investigate the reasons behind such behaviour. The initial findings of a sample of cases suggests that, in most cases, studies were performed for other regulatory purposes.

The registrants continue using read-across approaches and 995 dossiers (15 % of the total dossier submissions) contained *in vitro* or *in vivo* information solely on read-across substances. For the 995 dossiers analysed, 863 contained solely read-across *in vivo* data (13 % of 6 641 dossiers analysed), 92 dossiers contained solely *in vitro* and *in vivo* read-across data (1.4 % of 6 641 dossiers analysed) and 40 dossiers contained solely *in vitro* read-across data (0.6 % of 6 641 dossiers analysed). It is to 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.

Registrants are still making use of QSARs to a limited extent. In the majority of cases, registrants have used QSARs 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 QSAR estimations. ECHA notes that QSAR estimations could be more helpful when identifying substances requiring classification, but less useful to provide alerts on non-irritant substances.

Table 6.1: Type of dossiers submitted for eye irritation endpoint (6 641 substances assessed)[a]

	Number of dossiers	% of total dossier number	Dossier and ESR ratio[b]
Dossiers with only *in vitro*[c]	476	7.2	1:1
Dossiers with only in vitro on registered substance	*435*	*6.6*	*1:1*
Dossier with only in vitro read-across data	*40*	*0.6*	*1:1.5*
Dossiers with *in vitro* and *in vivo*[d]	803	12.1	1:1 in vitro 1:2.5 in vivo
Dossiers with in vitro and old in vivo[e]	*214*	*3.2*	*1:1 in vitro* *1:3 in vivo*
Dossiers with in vitro and old read-across in vivo[f]	*158*	*2.4*	*1:2 in vitro* *1:2 in vivo*
Dossiers with in vitro and new in vivo[e]	*342*	*5.2*	*1:1 in vitro* *1:1 in vivo*
Dossiers with in vitro and new read-across in vivo[g]	*88*	*1.3*	*1:2 in vitro* *1:5 in vivo*
Dossiers with old *in vivo*[e]	3 643	54.9	1:3.5
Dossiers with solely old *in vivo* read-across[f]	771	11.6	1:3
Dossiers with new *in vivo* for the registered substance[e]	289	4.3	1:1
Dossiers with new *in vivo* read-across data[g]	124	1.9	1:1
Dossiers with only QSARs, or estimations by calculation	79	1.2	1:1
Dossiers with only waiving statements	453	6.8	

a From the pool, duplicate dossiers for one substance were removed i.e. the number of dossiers analysed reflects the number of substances.

b These ratios are approximate values for illustrative purposes.

c The breakdown of use of in vitro methods reported below.

d The breakdown of use of in vitro and in vivo methods reported below.

e Substances contain at least one in vivo study performed with the registered substance, but may also contain studies performed with a read-across substance, QSARs and waiving statements.

f In the dossier, all in vivo studies have been performed with a read-across substance. They may also contain ESRs for QSARs and waiving statements.

g In the dossier, at least one new in vivo study has been performed with a read-across substance, but may also contain old read-across studies. They may also contain ESRs for QSARs and waiving statements.

Table 6.2: Breakdown of information provided per Annex (Annex VII 1 283, Annex VIII 767, Annex IX 2 205 and Annex X 2 386 substances evaluated)[a]

	Annex VII number of dossiers	% of total dossier number	Annex VIII number of dossiers	% of total dossier number	Annex IX number of dossiers	% of total dossier number	Annex X number of dossiers	% of total dossier number
Dossiers with only *in vitro*[c]	287	22.4	48	6.3	107	4.8	34	1.4
Dossiers with only in vitro on registered substance	*272*	*21.2*	*45*	*5.9*	*87*	*3.9*	*32*	*1.3*
Dossiers with only in vitro on read-across substance	*15*	*1.2*	*3*	*0.4*	*20*	*0.9*	*2*	*0.1*
Dossiers with *in vitro* and *in vivo*[d]	82	6.4	107	13.9	377	17.1	237	9.9
Dossiers with in vitro and old in vivo[e]	*24*	*1.9*	*26*	*3.4*	*75*	*3.4*	*89*	*3.7*
Dossiers with in vitro and old read-across in vivo[f]	*22*	*1.7*	*20*	*2.6*	*76*	*3.5*	*40*	*1.7*
Dossiers with in vitro and new in vivo[e]	*33*	*2.6*	*48*	*6.3*	*175*	*7.9*	*86*	*3.6*
Dossiers with in vitro and new read-across in vivo[g]	*3*	*0.2*	*12*	*1.6*	*51*	*2.3*	*22*	*0.9*
Dossiers with old *in vivo*[e]	668	52.1	428	55.8	1 078	48.9	1 469	61.6
Dossiers with solely old *in vivo* read-across[f]	65	5.1	65	8.4	297	13.5	344	14.4
Dossiers with new *in vivo* for the registered substance[e]	56	4.4	47	6.3	118	5.4	68	2.9
Dossiers with new *in vivo* read-across[g]	19	1.5	13	1.7	54	2.4	39	1.6
Dossiers with only QSARs, or estimations by calculation	16	1.2	11	1.4	20	0.9	32	1.3
Dossiers with only waiving statements	90	7.0	48	6.2	153	6.9	162	6.8

a From the pool, duplicate dossiers for one substance were removed i.e. the number of dossiers analysed reflects the number of substances.

c The breakdown of use of in vitro methods reported below.

d The breakdown of use of in vitro and in vivo methods reported below.

e Substances contain at least one in vivo study performed with the registered substance, but may also contain studies performed with a read-across substance, QSARs and waiving statements.

f In the dossier, all in vivo studies have been performed with a read-across substance. They may also contain ESRs for QSARs and waiving statements.

g In the dossier, at least one new in vivo study has been performed with a read-across substance, but may also contain old read-across studies. They may also contain ESRs for QSARs and waiving statements.

Appendix 7. 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 *in vitro*/*in chemico* skin sensitisation test methods that are currently adopted by the EU/OECD for the three key events as described in the adverse outcome pathway (AOP) are the following: direct peptide reactivity assay (DPRA, EU B.59/OECD TG 442C), keratinosens™ assay (EU B.60/OECD TG 442D) and h-CLAT assay (OECD TG 442E).

Several *in vitro* assay are in different validation stages or under regulatory adoption. The DPRA assay aims to provide information on the molecular initiating event (key event 1), i.e. protein biding. The keratinosensTM assay aims to provide information on inflammatory responses in keratinocytes (key event 2) where activity of the Keap1-Nrf2-ARE pathway is measured. The h-CLAT assay aims to provide information on dendritic cell activation (key event 3) where specific cell surface marker expression is measured by using a human monocytic leukemia cell line as an alternate model to dendritic cells.

As the current adopted methods are not meant to be used as standalone methods and only examine one key event of the skin sensitisation AOP, information from more than one key event is needed to conclude on skin sensitisation potential. Besides the adopted methods, a number of other *in vitro* methods have undergone or are currently under validation. 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. Some of the methods under evaluation are designed for potency assessment.

The *in vivo* 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.

The murine local lymph node assay (LLNA) is the first choice method for new *in vivo* testing, and another test should only be chosen in exceptional circumstances. Its use has to be justified.

The standard information requirements for this endpoint were amended on 27 September 2016 and entered into force on 11 October 2016. Data on skin sensitisation are required for substances produced or imported at or above 1 tpa, and hence should be in all the registrations considered for the purpose of this report.

Before the information requirement was amended, *in vivo* testing was the standard information requirement for all substances. The main change to the information requirement was that when new information needs to be generated, the testing has to start with non-animal test methods (*in vitro*/*in chemico*) addressing three key events from the skin sensitisation AOP i.e. molecular interaction with skin proteins, inflammatory responses in keratinocytes and activation of dendritic cells (REACH Annex VII, Section 8.3.1).

New *in vivo* testing must only be conducted if the *in vitro*/*in chemico* test methods are not applicable for the substance or the results of those test methods do not allow classification and risk assessment as specified in REACH Annex VII, Section 8.3 (REACH Annex VII, Section 8.3.2). The second change was that irrespective of how the information has been generated, for skin sensitising substances the potential of the substance to produce significant sensitisation in humans (Cat. 1A of CLP) needs to be assessed (REACH Annex VII, Section 8.3).

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 Annex VII to REACH (such as pH).

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, 102 *in vitro* ESR entries covering 50 dossiers were submitted by the end of March 2016. In 15 of the 50 dossiers, the skin sensitisation endpoint was covered solely with *in vitro*/*in chemico* studies. In 35 of the 50 dossiers, *in vitro*/*in chemico* studies were used together with additional information such as *in vivo* data (registered substance or read-across).

In the majority of 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 compared to the last report to fulfil the information requirements for this endpoint.

The quality of the submitted information has not been evaluated for this report. Due to the recent adoption of *in vitro*/*in chemico* test guidelines and the changes in the information requirements, it is anticipated that the use of non-animal approaches will increase substantially in the near future.

Appendix 8. Data availability table

The figures are based on data submitted by registrants and hence are not absolute. From manual checks for some endpoints, it became obvious that the computational analysis may overestimate the numbers and that not all possible variations of how registrants complete their registrations can be fully examined using IT tools. ECHA considers taking actions on some of these findings where the generation of new data may not have been in line with REACH. For the endpoints: skin and eye irritation, the numbers have been manually verified.

For this report, internationally recognised test guidelines are those listed in the OECD harmonised templates (OHT, 2016 edition) for analysed endpoints. If a test guideline was mentioned, but did not match the OECD harmonised templates study list it was flagged as "other". In addition, if a guideline was recognised but not considered as fully equivalent to the OECD guideline, it is listed as "generic test name (other)" (e.g. Acute oral toxicity (other)). The studies without any information on the guideline were not included in this analysis.

Table 8.1: Detailed results for data availability by UES analysis. NES – new experimental studies; OES – old experimental studies.

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
Bioaccumulation	OECD 305 and equivalent	*in vivo*	16	52	11	49	6	31	5	10	38	142	180
	OECD 305 B	*in vivo*	2	5	1	1	2	3	0	1	5	10	15
	OECD 305 C	*in vivo*	0	57	2	43	0	13	0	14	2	127	129
	OECD 305 D	*in vivo*	0	1	0	0	0	1	0	0	0	2	2
	OECD 305 E	*in vivo*	2	16	1	9	1	0	0	0	4	25	29
	Other	*n/a*	3	37	4	31	3	9	0	2	10	79	89
Subtotal			**23**	**168**	**19**	**133**	**12**	**57**	**5**	**27**	**59**	**385**	**444**
Short-term fish	OECD 203 and equivalent	*in vivo*	229	1 226	453	855	206	435	116	322	1 004	2 838	3 842
	OECD 204	*in vivo*	5	91	11	25	8	9	1	8	25	133	158
	OPPTS 850.1085	*in vivo*	0	1	0	3	0	0	0	0	0	4	4
	OECD 236: Fish embryo test (FET)	*in vivo*	3	0	1	0	0	1	0	0	4	1	5
	Other	*n/a*	17	532	2	243	5	86	3	71	27	932	959
Subtotal			**254**	**1 850**	**467**	**1 126**	**219**	**531**	**120**	**401**	**1 060**	**3 908**	**4 968**
Long-term fish	OECD 210 and equivalent	*in vivo*	43	87	25	32	16	18	7	6	91	143	234
	OECD 212 & EU C.15	*in vivo*	14	12	7	2	1	0	3	2	25	16	41
	OECD 215 & EU C.14	*in vivo*	6	22	6	4	1	1	1	0	14	27	41
	EPA OPP 72-5 & OPPTS 850.1500	*in vivo*	1	10	0	0	0	0	0	0	1	10	11
	Other	*n/a*	11	65	1	9	1	2	0	2	13	78	91
Subtotal			**75**	**196**	**39**	**47**	**19**	**21**	**11**	**10**	**144**	**274**	**418**
Birds	OECD 205 and equivalent	*in vivo*	1	31	0	20	2	13	0	2	3	66	69
	OECD 206 and equivalent	*in vivo*	3	13	0	8	3	10	0	2	6	33	39
	OECD 223 and equivalent	*in vivo*	0	21	0	11	2	10	0	1	2	43	45
	OTS 797.2175	*in vivo*	0	2	0	0	0	0	0	0	0	2	2
	Other	*n/a*	0	7	0	1	0	0	0	0	0	8	8

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
Subtotal			**4**	**74**	**0**	**40**	**7**	**33**	**0**	**5**	**11**	**152**	**163**
Toxicokinetics	n/a	*n/a*	34	326	44	114	23	54	3	20	104	514	618
Acute toxicity	OECD 401: Acute oral toxicity	*in vivo*	2	1 188	2	897	3	263	12	336	19	2 684	2 703
	OECD 420: Acute oral toxicity - fixed dose	*in vivo*	12	56	16	28	4	13	18	18	50	115	165
	OECD 423: Acute oral toxicity - toxic class method	*in vivo*	53	107	98	118	45	60	110	103	306	388	694
	OECD 425: Acute oral toxicity - up and down procedure	*in vivo*	23	26	23	18	2	10	4	6	52	60	112
	Acute oral toxicity (other)	*n/a*	99	428	196	419	89	254	201	318	585	1 419	2 004
	OECD 403: Acute inhalation toxicity	*in vivo*	65	559	43	247	19	72	17	87	144	965	1 109
	OECD 436: Acute inhalation toxicity- toxic class method	*in vivo*	30	1	43	3	10	1	5	0	88	5	93
	Acute inhalation toxicity (other)	*n/a*	31	117	24	88	15	28	10	11	80	244	324
	OECD 402: Acute dermal toxicity	*in vivo*	121	588	249	442	77	238	49	190	496	1 458	1 954
	OECD 434: Acute dermal toxicity- fixed dose	*in vivo*	1	15	2	6	2	3	0	0	5	24	29
	Acute dermal toxicity (other)	*n/a*	38	135	115	127	62	90	27	55	242	407	649
Subtotal			**475**	**3 220**	**811**	**2 393**	**328**	**1 032**	**453**	**1 124**	**2 067**	**7 769**	**9 836**
Skin irritation / corrosion	OECD 404: Acute dermal irritation/ corrosion	*in vivo*	117	1 411	195	937	83	373	107	489	502	3 210	3 712
	OECD 430: Transcutaneous electrical resistance	*in vitro*	2	8	3	5	0	1	10	3	15	17	32
	OECD 431: Human skin model test	*in vitro*	133	79	184	24	50	17	126	18	493	138	631
	OECD 435: Membrane barrier test	*in vitro*	11	15	23	8	7	2	13	3	54	28	82
	OECD 439: Skin irritation	*in vitro*	157	10	338	3	94	3	251	9	840	25	865
	In vitro skin irritation/corrosion (other)	*in vitro*	2	0	11	1	2	0	1	0	16	1	17

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
	Other	*n/a*	38	400	76	327	43	160	82	213	239	1 100	1 339
Subtotal			**460**	**1 923**	**830**	**1 305**	**279**	**556**	**590**	**735**	**2 159**	**4 519**	**6 678**
Eye irritation	OECD 405: Acute eye irritation/corrosion	*in vivo*	187	1 088	316	739	115	329	116	434	734	2 590	3 324
	OECD 437: Bovine corneal opacity and permeability	*in vitro*	105	14	249	5	70	3	220	13	644	35	679
	OECD 438: Isolated chicken eye test	*in vitro*	12	11	29	4	15	3	31	0	87	18	105
	OECD 460: Fluorescein leakage test	*in vitro*	2	1	0	0	0	0	0	0	2	1	3
	OECD 491: In vitro short-time exposure	*in vitro*	1	0	0	0	0	0	0	0	1	0	1
	OECD 492: Reconstructed human cornea-like epithelium	*in vitro*	56	4	90	0	23	3	54	11	223	18	241
	In vitro eye irritation (other)	*in vitro*	38	52	42	42	22	19	29	18	131	131	262
	Other	*n/a*	122	309	158	265	77	152	126	198	483	924	1 407
Subtotal			**523**	**1 479**	**884**	**1 055**	**322**	**509**	**576**	**674**	**2 305**	**3 717**	**6 022**
Skin sensitisation	OECD 406 and equivalent	*in vivo*	44	822	69	549	22	268	31	355	166	1 994	2 160
	OECD 429: Local lymph node assay (LLNA)	*in vivo*	186	186	332	132	97	92	276	133	891	543	1 434
	Local lymph node assay (other)	*in vivo*	60	41	99	66	43	49	102	38	304	194	498
	OECD 442C: Direct peptide reactivity assay (DPRA)	*in chemico*	3	0	14	0	7	0	10	0	34	0	34
	OECD 442D: ARE-Nrf2 luciferase test method	*in vitro*	0	0	2	0	0	0	2	0	4	0	4
	OECD 442E: Human cell line activation test (h-CLAT)	*in vitro*	1	0	1	0	1	0	6	0	9	0	9
	In vitro skin sensitisation (other)	*in vitro*	14	0	22	0	9	0	10	0	55	0	55
	Other	*in vivo*	79	349	55	205	15	65	7	30	156	649	805
Subtotal			**387**	**1 398**	**594**	**952**	**194**	**474**	**444**	**556**	**1 619**	**3 380**	**4 999**

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
Repeated dose toxicity	OECD 408: Repeated dose toxicity 90-day oral	*in vivo*	79	349	55	205	15	65	7	30	156	649	805
	Repeated dose toxicity 90-day oral (other)	*in vivo*	24	55	16	32	7	19	1	5	48	111	159
	OECD 409: Repeated dose toxicity 90-day oral non-rodents	*in vivo*	1	42	1	29	1	22	0	6	3	99	102
	Repeated dose toxicity 90-Day oral non-rodents (other)	*in vivo*	1	5	1	11	2	4	0	0	4	20	24
	OECD 407: Repeated dose toxicity 28-day oral	*in vivo*	64	290	168	272	66	225	49	155	347	942	1 289
	Repeated dose toxicity oral (other)	*in vivo*	6	94	16	64	7	45	6	25	35	228	263
	OECD 413: Repeated dose toxicity 90-day inhalation	*in vivo*	23	227	9	40	4	14	1	3	37	284	321
	Repeated dose toxicity 90-day inhalation (other)	*in vivo*	9	30	4	8	2	2	0	1	15	41	56
	OECD 412: Repeated dose toxicity 28-day inhalation	*in vivo*	44	209	21	67	9	10	5	13	79	299	378
	Repeated dose toxicity inhalation (other)	*in vivo*	0	0	0	0	0	0	0	0	0	0	0
	OECD 410: Repeated dose toxicity 90-day dermal	*in vivo*	2	50	1	17	0	6	2	2	5	75	80
	Repeated dose toxicity 90-day dermal (other)	*in vivo*	0	8	0	5	0	0	0	0	0	13	13
	OECD 410: Repeated dose toxicity 21/28-day dermal	*in vivo*	5	116	6	18	1	14	4	6	16	154	170
	Repeated dose toxicity 21/28-day dermal (other)	*in vivo*	0	13	0	7	0	6	0	1	0	27	27
	Repeated dose toxicity dermal (other)	*in vivo*	1	16	1	5	3	5	0	1	5	27	32
	OECD 452: Chronic toxicity studies	*in vivo*	0	54	0	29	0	8	0	4	0	95	95

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
	Chronic toxicity studies (other)	*in vivo*	2	10	0	9	3	9	0	2	5	30	35
	OECD 453: Combined chronic toxicity/ carcinogenicity	*in vivo*	8	239	5	43	2	15	0	11	15	308	323
	Combined chronic toxicity/carcinoge-nicity (other)	*in vivo*	0	14	1	4	2	2	0	0	3	20	23
	OECD 419: Repeated dose toxicity 28-day delayed neurotoxicity	*in vivo*	0	1	0	1	0	1	0	1	0	4	4
	Repeated dose toxicity 28-day delayed neurotoxicity (other)	*in vivo*	0	1	0	0	0	0	0	0	0	1	1
	OECD 424: Neurotoxicity study in rodents	*in vivo*	1	4	1	1	0	0	0	1	2	6	8
Subtotal			**270**	**1 827**	**306**	**867**	**124**	**472**	**75**	**267**	**775**	**3 433**	**4 208**
Genotoxicity in vitro	OECD 471,472: Gene mutation study in bacteria	*in vitro*	265	1 291	357	973	190	399	367	582	1 179	3 245	4 424
	OECD 476, 490: Gene mutation study in mammalian cells	*in vitro*	337	446	466	199	221	84	60	58	1 084	787	1 871
	OECD 473: Cytogenicity/chromosome aberration study in mammalian cells	*in vitro*	184	514	251	323	114	171	76	143	625	1 151	1 776
	OECD 487: Cytogenicity/micronucleus study	*in vitro*	24	5	57	5	30	4	14	2	125	16	141
	OECD 479, 481, 482: DNA damage and / or repair study	*in vitro*	1	162	1	70	0	26	1	20	3	278	281
	B.21: Transformation study in mamma-lian cells	*in vitro*	0	21	0	8	0	2	1	2	1	33	34
	Other	*in vitro*	30	278	62	165	33	101	45	150	170	694	864
Subtotal			**841**	**2 717**	**1 194**	**1 743**	**588**	**787**	**564**	**957**	**3 187**	**6 204**	**9 391**

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
Genotoxicity in vivo	OECD 475: Mammalian somatic cell study: cytogenicity/bone marrow chromosome aberration	*in vivo*	4	139	2	45	1	8	2	8	9	200	209
	Mammalian somatic cell study: cytogenicity/bone marrow chromosome aberration (other)	*in vivo*	1	8	0	3	1	1	2	0	4	12	16
	OECD 474: Mammalian somatic cell study: cytogenicity/erythrocyte micronucleus	*in vivo*	52	411	70	273	36	125	20	103	178	912	1 090
	Mammalian somatic cell study: cytogenicity/erythrocyte micronucleus (other)	*in vivo*	13	39	24	16	16	11	11	7	64	73	137
	OECD 485, 483, 478: Mammalian germ cell study: cytogenicity/chromosome aberration	*in vivo*	0	61	1	26	0	10	0	5	1	102	103
	Mammalian germ cell study: cytogenicity/chromosome aberration (other)	*in vivo*	0	0	0	2	0	2	0	0	0	4	4
	OECD 489, 486: Mammalian cell study: DNA damage and/or repair	*in vivo*	5	47	6	31	6	16	5	15	22	109	131
	Mammalian cell study: DNA damage and/or repair (other)	*in vivo*	0	11	0	6	0	0	0	1	0	18	18
	OECD 488: Mammalian somatic and germ cell study: gene mutation	*in vivo*	1	0	0	0	0	0	0	0	1	0	1
	OECD 484: Mammalian germ cell study: gene mutation	*in vivo*	0	4	0	0	0	1	0	0	0	5	5
	Mammalian germ cell study: gene mutation (other)	*in vivo*	0	11	0	6	0	0	0	1	0	18	18
	B.24: In vivo mammalian somatic cell study: gene mutation	*in vivo*	0	1	0	0	0	0	0	0	0	1	1
	OECD 477: In vivo insect germ cell study: gene mutation	*in vivo*	0	25	0	11	0	3	0	2	0	41	41
	Insect germ cell study: gene mutation (other)	*in vivo*	0	2	0	0	0	0	0	0	0	2	2
	Other	*in vivo*	7	56	5	28	4	26	2	9	18	119	137
Subtotal			**83**	**815**	**108**	**447**	**64**	**203**	**42**	**151**	**297**	**1 616**	**1 913**

Endpoint	Test Guideline	*in vitro/in vivo*	Annex X		Annex IX		Annex VIII		Annex VII		NES subtotal	OES subtotal	Total
			NES	OES	NES	OES	NES	OES	NES	OES			
Carcinogenicity	OECD 451: Carcinogenicity studies	*in vivo*	9	188	0	54	0	22	0	11	9	275	284
	Other	*n/a*	4	64	0	34	2	12	0	7	6	117	123
Subtotal			**13**	**252**	**0**	**88**	**2**	**34**	**0**	**18**	**15**	**392**	**407**
Toxicity to reproduction	OECD 415: One-generation reproduction toxicity study	*in vivo*	7	68	8	29	4	10	0	6	19	113	132
	OECD 416: Two-generation reproduction toxicity study	*in vivo*	15	120	8	35	0	5	0	1	23	161	184
	OECD 443: Extended one-generation reproductive toxicity study	*in vivo*	0	0	0	0	0	0	0	0	0	0	0
	OECD 421: Reproduction/developmental toxicity screening	*in vivo*	59	93	129	47	139	16	13	6	340	162	502
	OECD 422: Combined repeated dose toxicity 28-day with the reproduction/developmental toxicity screening	*in vivo*	122	174	303	105	142	24	20	20	587	323	910
	Combined repeated dose toxicity 28-day with the reproduction/developmental toxicity screening (other)	*in vivo*	2	2	8	2	6	0	1	1	17	5	22
	Reproduction and fertility effects (other)	*n/a*	15	117	11	51	5	28	0	10	31	206	237
Subtotal			**220**	**574**	**467**	**269**	**296**	**83**	**34**	**44**	**1 017**	**970**	**1 987**
Developmental toxicity	OECD 414: Pre-natal developmental toxicity study	*in vivo*	114	614	73	211	14	60	9	29	210	914	1 124
	Pre-natal developmental toxicity study (other)	*in vivo*	70	169	50	99	24	37	5	11	149	316	465
	Preliminary developmental toxicity screen	*in vivo*	1	42	4	3	3	4	0	1	8	50	58
	OECD 426: Developmental neurotoxicity study	*in vivo*	0	3	1	0	0	0	0	0	1	3	4
	Developmental neurotoxicity study (other)	*in vivo*	1	3	0	0	0	0	0	0	1	3	4
Subtotal			**186**	**831**	**128**	**313**	**41**	**101**	**14**	**41**	**369**	**1 286**	**1 655**
Total			**3 848**	**17650**	**5 891**	**10892**	**2 518**	**4 947**	**2 931**	**5 030**	**15188**	**38519**	**53 707**

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