ECHA
EUROPEAN CHEMICALS AGENCY

Practical guide

How to use alternatives to animal testing to fulfil your information requirements for REACH registration

Version 2.0 – July 2016

Version	Changes
Version 1.0	First versions of practical guides 1, 2, 4, 5, 6 and 10
Version 2.0	Compilation of the previous practical guides (PGs), covering the various areas. The update includes the following: Chapter 2: (PG10) How to avoid unnecessary testing on animals. Chapter 3.3: (PG4) How to report data waiving Chapter 3.1 and 4.1: (PG2) How to report Weight of Evidence Chapter 4.2: Separate update of PG5: How to report QSAR; called “How to use and report (Q)SARs” Chapter 4.3: (PG1) How to report *in vitro* data Chapter 4.4: (PG6) How to report read-across and categories

Practical Guide: How to use alternatives to animal testing to fulfil the information requirements for REACH registration

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1. Introduction

The REACH Regulation[1] requires new information on hazardous properties to be generated avoiding unnecessary animal testing where possible.

The purpose of this practical guide is to inform you about your obligations to avoid unnecessary testing on vertebrate animals, yet still ensure that you have sufficient information on the properties of your substances for classification and risk assessment. To this end, it explains the increasing opportunities for using alternatives to animal testing and how to report these correctly.

This practical guide also provides recommendations based on ECHA's experience so far with the registration and dossier evaluation processes. Note that the information given in this guide does not describe the requirements to pass the completeness check of your registration. This is described in the Annex 2 of the Manual "How to prepare registration and PPORD dossiers".

You may also want to consult the *Guidance on information requirements and chemical safety assessment* (including chapters R2, R3, R4, R5, R6 and R7). These more detailed guidance documents provide examples and explanations of the concepts introduced here.

Finally, ECHA also provides information in its *Practical Guide for SME Managers and REACH coordinators* (Chapter 2.2).

1.1 Who should read this guide?

This guide is aimed especially at manufacturers and importers of substances (and their only representatives) and should be especially useful to small and medium-sized enterprises (SMEs) who have responsibilities under the REACH or CLP regulations.

It is also useful for contract research organisations and consultants providing services to registrants. It may help you to make decisions on your registrations and to assess the advice you may be provided by other parties. Furthermore, companies outside the European Union (EU) exporting chemicals to the EU may also find the document useful.

1.2 Essentials

The present practical guide can be summarised in a few key messages:

1. Follow the "four steps" for fulfilling information requirements:
   (i) Gather and share existing information;
   (ii) Consider the information needs;
   (iii) Identify information gaps; and
   (iv) Generate new data or propose a testing strategy.
2. Share data with other (potential) registrants (in SIEFs for phase-in substances) or previous registrants. Request the existing information involving tests on vertebrate animals from the previous registrants.
3. In some cases, you can rely on data waiving if it is justified in accordance with REACH requirements (Annexes VII-X, second column and/or Annex XI).

[1] Regulation EC No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

4. If new data needs to be generated, animal testing is the last resort. In some cases, animal testing requires prior approval by ECHA. As you are obligated to consider alternative methods, you need to keep records of your considerations to support your conclusion as to why it is necessary to generate information using vertebrate animals. You may be requested to submit your consideration of alternative methods.

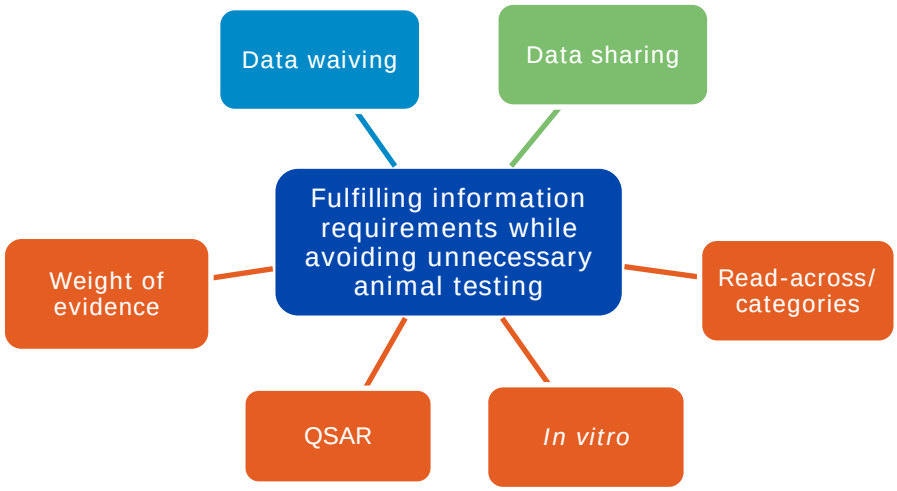
5. Alternative methods can be used, instead of direct testing, to draw conclusions for several information requirements. The alternative methods outlined in REACH are a weight of evidence approach, *in vitro* methods, quantitative structure-activity relationship (QSAR) models and read-across/categories. However, the legal requirements vary according to the specific endpoints. The level of information should be equivalent to that produced by the standard tests.

6. Document that the formal preconditions for the use of alternative data are fulfilled, including that they have been obtained with validated methods and that the results are adequate for classification and labelling and/or risk assessment.

7. Good quality dossiers are required. Note that if ECHA identifies inadequate data, the missing information can be requested at a later stage.

More detailed information on integrated testing strategies to fulfil the information requirements is available (see *Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance*).

Figure 1: Relationship between the standard information requirements (IRs) and possible alternatives to (animal) testing



2. Your general obligations

Assessing hazards and risks of your substances: the overall purpose of both the REACH and the CLP[2] regulations is to ensure a high level of protection of human health and the environment.

To achieve this, adequate information on the properties of chemical substances is needed to decide on their classification, labelling and risk assessment. Therefore, REACH requires you to register your substances and fulfil the information requirements as stipulated in Articles 10 and 12 in conjunction with Annexes VI to XI to the REACH Regulation.

The CLP Regulation does not require new studies[3]. Instead, you have to obtain and evaluate all the available relevant information to classify your substances and mixtures. In practice, this means that many substances can be classified on the basis of data obtained while preparing to register under REACH.

Sharing of results of tests involving vertebrate animals: as a primary means of avoiding unnecessary testing on animals, registrants are obligated under REACH to share the results of tests involving vertebrate animals with their co-registrants of the same substance and to create a joint submission.

REACH requires registrants to first gather and share existing information, consider the information needs, then identify information gaps and, only then, if necessary, generate new data or propose a testing strategy.

Depending on the substance and the endpoint, the conclusion may be that the existing information on the hazards to human health and the environment is inadequate and that new information will need to be generated.

Information on properties of substances may be generated by means other than tests, provided that the conditions set out in REACH are met. However, in many cases, additional testing is the only way to fill the information gaps.

Testing on vertebrate animals only as a last resort: REACH specifically requires information to be generated whenever possible by means other than vertebrate animal tests. In other words, testing on animals is only allowed as a last resort when all other data sources have been exhausted.

Alternative test methods such as *in vitro* tests are continuously being developed and REACH standard information requirements are consequently being adapted. Yet, many of the information requirements, especially for the chemicals registered in high tonnages, rely on standard test methods using vertebrate animals as a model to predict the effects of chemicals on humans and the environment. However, there are other means to assess the properties of substances even for these endpoints, such as read-across and grouping.

Where a new animal test proves necessary, legislation requires that scientifically-sound approaches to the implementation of the 3Rs – reduction, refinement or replacement of animal use – are used.

The least severe test that uses the fewest animals needs to be employed and conducted in a

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[2] Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
[3] Except for physicochemical properties which is outside the scope of this document

way that causes the least pain, suffering, distress and lasting harm. The test has to be done in compliance with the provisions of Directive 2010/63/EU on the protection of animals used for scientific purposes and using acknowledged methods laid down in the Test Methods Regulation (EC No 440/2008).

Note that for all the studies required to fulfil REACH Annex IX or X requirements and for certain studies following up Annex VIII requirements, you have to submit testing proposals and receive approval from ECHA before you are allowed to perform the test.

Under the REACH evaluation processes, ECHA examines any testing proposals and may also select your dossier for compliance check. If ECHA identifies concerns that available alternative methods seem not to have been used while examining your dossier, the Agency can require you to clarify the issue. If the concerns are not addressed, ECHA may inform the Member State authorities of the potential incompliance. You should therefore document your justifications as to why it was necessary to generate new animal studies.

Finally, you are obligated to update your dossier, without undue delay, with new information and studies that become available.

3. Fulfil your information requirements – four-step process

As a registrant, you have to obtain data on your substances as specified in Annexes VI-X to REACH. Annex VI to REACH provides a basic four-step procedure for fulfilling the information requirements. Note that these steps are not necessarily consecutive. I

n practice, this is an iterative process which is also illustrated in Figure 2 below. This is an overview of the recommended steps to define a correct strategy and to ensure that unnecessary animal testing and duplicate tests are avoided.

A comparable process can be used for the classification of substances, although under the CLP Regulation, you are not obligated to conduct new studies.

Step 1 – Gather and share existing information

Gathering and sharing all available existing information is the first step in the process of fulfilling your information requirements. This is further explained in Chapters 3.1 and 3.2 below.

Step 2 – Consider information needs

From Annexes VII-X to REACH, you need to identify the standard information requirements on the intrinsic properties of your substance, applying to the tonnage you manufacture or import, because you need to comply with these information requirements. You also need to identify from Annex VI all necessary information on substance identity.

You should also already at this stage consider any potential options for adapting or waiving the information requirements as detailed in Column 2 of Annexes VII-X (specific rules per endpoint), and in the sections of Annex XI (general criteria for adaptation of the information requested). These will be discussed further below (see Chapters 3.3 and 4).

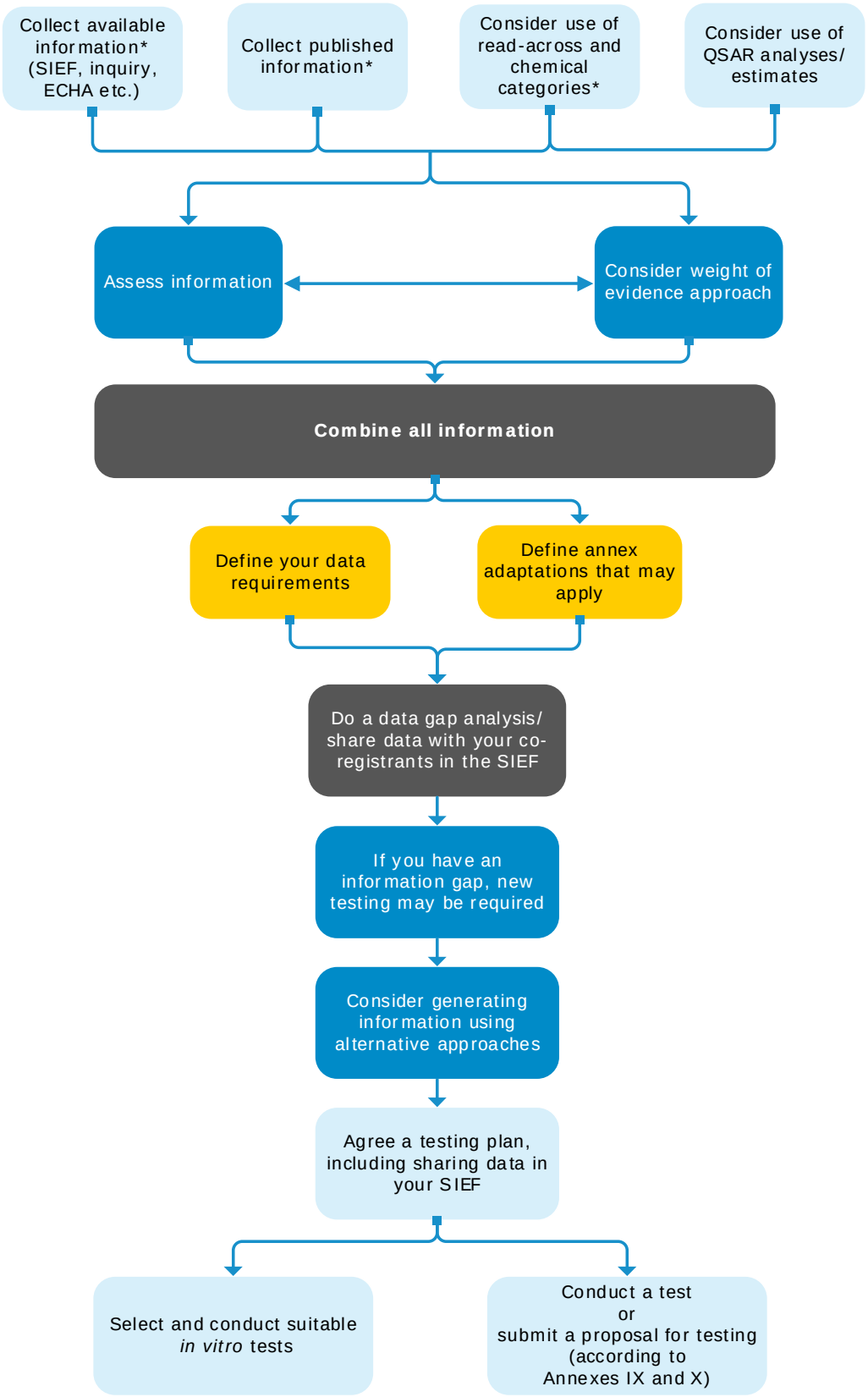
Step 3 – Identify information gaps

After collecting all available relevant and reliable information in Step 1, you need to compare the information needs for your substance identified in Step 2, to see if there are any gaps in the data required.

Step 4 – Generate new data or propose a testing strategy

If a data gap is identified in Step 3, then you must generate new information. This is further explained in Chapter 3.4 below.

Figure 2: Overview of the iterative process of gathering information, to avoid unnecessary animal testing



* You must have the right to use the information.

3.1 Using existing information

You should collect all relevant available information on the intrinsic properties of your substance such as physical-chemical properties, environmental fate and toxicity and mammalian toxicity, as well as use and exposure, regardless of whether information on a given endpoint is required at the specific tonnage level.

This includes any available, adequate and reliable information, from you or other data owners and sources:

- Existing data on the substance whether from testing or other sources (e.g. scientific publications);
- Manufacture and all uses of the substance, information on exposure to humans and the environment and any related risk management measures;
- Data on analogous substances if "read across" or insertion in a "chemical category" is possible (consider contacting SIEFs with related substances);
- (Q)SAR estimated results if suitable models are available;
- Any other information, which could support a weight of evidence approach to fill data gaps for particular endpoints, if this is appropriate.

REACH requires that you include all information that is relevant and available to you in the technical dossier. As a minimum, you need to provide the standard information required in REACH Annexes VII to X, as relevant to the tonnage of your registration.

In practice, after gathering and assessing all existing information, you have to select the information that is **relevant**, **adequate** and **reliable**. Based on this assessment, you need to submit any information that has been useful in fulfilling your requirements for each specific endpoint of the substance and report all data that has been necessary to demonstrate its safe use. Although one data endpoint from a relevant, adequate and high quality study is in principle sufficient to fulfil an information requirement, the more data provided, the more robust the conclusions.

In REACH Annex XI Section 1.1, the use of existing data may be considered as a valid justification that testing is scientifically unjustified where the conditions stipulated are met. By using and correctly reporting existing data, you will contribute to avoiding unnecessary testing on animals. Appropriate reporting on existing information is also the basis for using alternatives such as weight of evidence (see Chapter 4.1) and read-across or grouping (see Chapter 4.4).

How should it be done?

General criteria for scoring the information

- The general criteria to score information are reliability, relevance and adequacy, and are described comprehensively in the Guidance on information requirements and chemical safety assessment, Chapter R.4. In short, these terms have been defined by Klimisch et al. (1997)[4] as follows:

[4] Klimisch H, Andreae M and Tillmann U (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology Volume 25 (1).

- **Relevance** - covering the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation.

- **Reliability** - evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. Reliability of data is closely linked to the reliability of the test method used to generate the data (see Section R.4.2 of the Guidance).

- **Adequacy** - defining the usefulness of data for hazard/risk assessment purposes. Where there is more than one study for each endpoint, the greatest weight is attached to the studies that are the most relevant and reliable. For each endpoint, robust summaries need to be prepared for the key studies.

Relevance of the information to identify the hazards and risks

The relevance of information means the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation. It is not limited to actual test data, but can cover other types of information.

When assessing relevance, you should consider:

- the relevance of the endpoint: the effects investigated in a study should be clearly related to the toxicity of the substance (e.g. physical effects, effects due to complicating factors are not relevant);

- the relevance of the test material: the test material should be equivalent to the registered substance;

- the relevance of the test method and conditions: conditions used should not vary too much from those of internationally approved test guidelines;

- the relevance of the alternative data: e.g. when using (Q)SAR, read across, categories or *in vitro* approaches, you should verify whether they are applicable for the substance (e.g. applicability domain of the (Q)SAR models, consistency of the category, relevance of the *in vitro* effects).

Reliability of the information to identify the hazards and risks

Relevant information must be reliable enough to be taken into account when identifying the hazards and risks, as indicated in the Guidance on information requirements and chemical safety assessment, Chapter R.4. You should only submit information if you have evidence that its content is relevant, reliable and adequate.

Hence, reliability is measured by the quality of the study, the method used, the reporting of the results and the conclusion. Therefore, the reliability of a test may result from the quality of the test report, the use of a standardised methodology and the way the experimental procedure and results are described.

To communicate on the reliability of a given study, you need to assign, for all information you provide in the technical dossier, a score according to the Klimisch scoring system:

1 = reliable without restrictions;
2 = reliable with restriction;
3 = not reliable;
4 = not assignable.

Adequacy of the information for the identification of the hazards and risks

The adequacy is essentially the usefulness of information for the purpose of hazard and risk assessment.

The information you submit also needs to be adequate for a particular hazard identification or risk characterisation; it should allow clear decision-making about whether the substance meets the criteria for classification and allow appropriate DNEL/PNEC values to be derived for risk assessment.

Quantity

In addition to the above, quantity is a criterion to be considered when assessing the strength of the evidence, especially when multiple sources of information are available to build a weight of evidence and to adapt the requested endpoint study. The overall weight of the evidence requires more than one piece of information. As indicated above, the more pieces of evidence that are available, in particular if contradictory pieces of information are encountered, the better.

Common data sources and their scoring

The following sources may yield useful information:

- Handbook information and databases
- Existing studies – old data
- Epidemiological studies and other human data
- (Q)SAR prediction
- *In vitro* and newly developed test methods
- Read-across

You have to verify that you have the right to use these data for the purpose of registration (see also the *Guidance on Data Sharing*).

Handbook information and databases

For well-studied chemicals, it may be acceptable to use values for physicochemical, toxicological and ecotoxicological parameters obtained from 'peer-reviewed' data. It is appropriate to assign these sources of peer-reviewed data a reliability score 2, 'valid with restrictions', when considering reliability, since it is assumed that a variety of data sources have been consulted, that the test methodology and identity of the test substance has been evaluated, and that a reliable and representative value for the endpoint has been selected. Whether such a review process has been conducted should be stated in the introduction to the handbook or contained in the summary information for an online database.

Useful reference books and data compilations containing peer-reviewed physiochemical data are given in the Guidance on information requirements and chemical safety assessment Chapter R7a (Table R.7.1-2).

Online databases, such as the participating databases on the OECD eChemPortal, are useful sources of data, particularly if they provide a reference for the value selected, and they serve as a source to highlight where further data are available. Remember that the original data source should be checked and referenced, rather than directly citing the database (or secondary data source without retrieving it) because these database sources are usually secondary data sources themselves.

When using data solely from multiple secondary sources, it is essential to construct a weight of evidence approach (see more details in Chapter 4.1) to establish that an appropriate value has been selected for the endpoint of interest. Generally, it is not acceptable to use a single, peer-reviewed secondary source with no further supporting evidence.

The technical dossier should present values taken from multiple authoritative data sources, in addition to the supporting data such as manufacturing data, reliable QSAR predictions, and/or data from sources that may not have been peer-reviewed.

Values for physico-chemical properties taken from material safety data sheets and all other company technical data can only be assigned a reliability score of 4 (i.e. not assignable), unless detailed information such as the experimental methodology and test substance are provided to enable the preparation of a (robust) study summary and an independent evaluation of the study reliability.

It is difficult to draw general conclusions regarding the reliability of each data source for an individual parameter. Reviewers need to ensure that the test substance identity, test method and result are reliable.

Existing studies – old data

There is no definition for an 'old study' but two distinctions can be made:

(i) whether the study has been conducted in compliance with the principles of good laboratory practice (GLP) provided for in Directive 2004/10/EC and studies that have been performed before the set-up of the GLP scheme (1987). The reliability of non-GLP studies should be assessed on a case-by-case basis with the Klimisch scoring system, and will highly depend on the quality of the study report;

(ii) whether the studies were performed according to the most recent Commission Regulation or OECD guidelines. The equivalence to the latest guideline should be considered on a case-by-case basis.

Although Annex XI to REACH clearly allows the use of existing studies, data from old studies that were not performed according to the current test guidelines may be less reliable or relevant, since the guideline followed may not be in line with the most recent ones. In particular, if fewer (or different) parameters were measured, reporting and quality assurance could be lacking. Hence, the reliability of such studies may be lower and as a result render them inadequate to be considered as key studies.

Nevertheless, these studies could be adequate within a weight of evidence approach or as supporting studies. To enable ECHA to evaluate these non-standard data, you should provide as much documentation as possible, including a detailed description of the study, its test method and test material and any deviations or abnormalities. If there is not enough information to document a complete robust study summary in IUCLID, the study would be better submitted as a supporting study.

Historical human data

According to Annex XI, Section 1.1.3 historical human data may be used to justify that testing is scientifically unjustified where the conditions stipulated are met.

Epidemiological data and other experience of human exposure, such as accidental poisoning or occupational exposure, clinical studies or case reports may be useful to include in a weight of evidence approach. Adequate and reliable documentation has to be provided concerning the criteria of Annex XI, Section 1.1.3 for assessing the adequacy of the data.

(Q)SAR prediction

Consideration of a valid (Q)SAR prediction may provide further evidence. Further details and guidance on the use of (Q)SAR data are available in the Practical Guide on "How to use and report (Q)SARs" and the Guidance on information requirements and chemical safety assessment, Chapter R.6.

In vitro and newly developed test methods

In vitro tests can be a source of evidence. Further details are given in Chapter 4.3 below.

Section 1.2 of Annex XI to REACH mentions that "newly developed test methods, not yet included in the test methods referred to in Article 13(3)", and which may be still in the pre-validated stage, could be considered within a weight of evidence approach. Information generated using *in vitro* methods can be useful for providing additional evidence that assists in explaining findings from *in vivo* tests. In particular, *in vitro* generated metabolism and kinetic data can aid in identifying the mode of action when combined with data from in vivo tests; such data can also assist in developing kinetic models. Remember that the *in vitro* data has to be reported in sufficient detail in the registration dossier to allow the relevance in the risk assessment to be evaluated.

Read-across

Consideration can be given to the use of information generated with an analogue chemical or as part of a chemical category (see the Guidance on information requirements and chemical safety assessment, Chapter R.6 and Chapter 4.4 below).

Expertise required

Administrative expertise	If available results of a test, including with the relevant Klimisch scores, can be used directly as input in the registration dossier.
Scientific expertise	If available results require Klimisch scores to be set or an interpretation, to conclude on a relevant value for further assessment.
Advanced scientific expertise	If multiple sources of evidence, e.g. from experimental data, can be used as alternatives to standard testing. Use of, scientific justification for, and reliable documentation of such data are subject to very specific rules. If other scientific data need to be negotiated with other registrants based on scientific outcomes of experimental or other data.

3.2 Data sharing

Sharing data is an obligation under REACH for registrants of the same substance. It is the primary means of avoiding unnecessary tests on animals. Hence, any existing studies using vertebrate animals conducted by one registrant must be shared for use by all co-registrants that need that information. It also means that any new animal studies that are needed for their own registration must also be agreed and shared among the co-registrants to prevent tests being duplicated.

Studies that do not involve vertebrate animal testing should also be shared to reduce the costs of registration.

Data sharing is also strongly encouraged among registrants of analogue substances (not part of your SIEF) to avoid unnecessary animal testing.

The data-sharing process is only briefly introduced here, as a more detailed description is available in the *Guidance on data sharing.*

Before registering jointly, registrants must discuss the sharing of data when a substance is manufactured or imported by more than one company.

There are two mechanisms for data sharing, independent from whether the substance has already been registered:

- For a phase-in (existing) substance that has been pre-registered: data sharing occurs within the substance information exchange forums (SIEFs);
- For a non-phase-in (new) substance and for a phase-in substance that has not been pre-registered: data sharing occurs after an inquiry.

How should it be done?

Registrants of the same substance must make every effort to make sure that the costs of sharing the information required for joint registration are determined in a fair, transparent and non-discriminatory way. All parties must fulfil their data-sharing and joint submission obligations in a timely manner. If parties cannot reach an agreement, ECHA can help to resolve data-sharing disputes. This should, however, be used as a last resort.

Advice on working with co-registrants is provided on ECHA's website.

Additional tips

Registrants may want to use data that is not owned by a SIEF member. In this case, an agreement from the data owner is needed. It is recommended that such an agreement is valid for all co-registrants including future ones. This would allow co-registrants to use the data without having to individually negotiate access to it.

3.3 Data waiving

What is it?

The REACH Regulation foresees that generating information required in Annexes VII-X may not be necessary or possible. In such cases, you are allowed to not provide (i.e. waive) the standard information for the endpoint. The criteria for waiving are outlined in REACH in Column 2 of Annexes VII-X, while criteria for adapting standard information requirements are describe in Annex XI.

Careful use of these options allows you to avoid unnecessary animal testing. Importantly, omitting testing on animals must not compromise the safe use of substances.

ECHA has noticed that testing has frequently been omitted based on inappropriate or insufficiently justified scientific arguments. According to REACH, every waiving to the standard information requirements you claim, must meet the relevant conditions set out either in column 2 of the Annexes VII-X, or in the relevant section of Annex XI. Furthermore, you need to provide a scientific and valid justification that supports your waiving of the testing for a specific endpoint, and you must document it clearly in the technical dossier and, where applicable, the chemical safety report.

In addition to the clear, well-documented and robust justification, you need to submit the supporting evidence so ECHA can independently assess their validity. A justification that is of poor quality or is insufficiently documented may lead to follow-up action from ECHA or Member States in cases where the safe use of a substance may be compromised.

Specific rules in column 2 of Annexes VII-X

Most endpoints have specific sets of conditions, part of column 2, under which the test may be:

(i) omitted;
(ii) replaced by other information (existing or to be generated), e.g. a short-term 28-day repeated dose toxicity study may be replaced with a reliable sub-chronic 90-day toxicity study;
(iii) provided at a later stage; or
(iv) adapted in another way (e.g. in Annex VIII, Section 8.5, for acute toxicity testing the choice of a second route of exposure will depend on the nature of the substance and the likely route of human exposure).

A test that is not already available may not be needed if it can be shown that certain criteria are met i.e. if the conditions specified in Column 2 to adapt the information requirement are fulfilled. There are a number of different possibilities depending on the information required:

- For example, if a justification is provided which shows the substance is spontaneously flammable in air at room temperature, testing for skin corrosion/irritation, serious eye damage/eye irritation (Annexes VII and VIII, Sections 8.1 and 8.2, respectively) and skin sensitisation (Annex VII, Section 8.3) (*in vitro* and *in vivo*) may be unnecessary.

- Another example is the case when an acute toxicity study (Annex VIII, Section 8.5) can generally be omitted if the substance is classified as corrosive to the skin (Category 1). Since mid-2016, a revision to the requirement for an acute dermal toxicity study (Annex VIII, Section 8.5) introduced additional adaptation possibilities, e.g. testing by the dermal route does not need to be conducted if the substance does not meet the criteria for classification for acute toxicity or STOT SE and further supporting information is provided.

- You do not need to conduct a sub-chronic toxicity study (90 days) if a reliable short-term toxicity study (28 days) is available and showing severe toxicity effects according to the criteria to classify the substance, as STOT RE, Category 1 or 2, and for which the observed NOAEL-28 days, with the application of an appropriate uncertainty factor, allows the extrapolation towards the NOAEL-90 days for the same route of exposure.

In all cases when Column 2 specific rules are used to omit a test, the conditions must be recorded in IUCLID, under the specific endpoint entry and using the appropriate reason from the pick-lists provided.

General rules in Annex XI

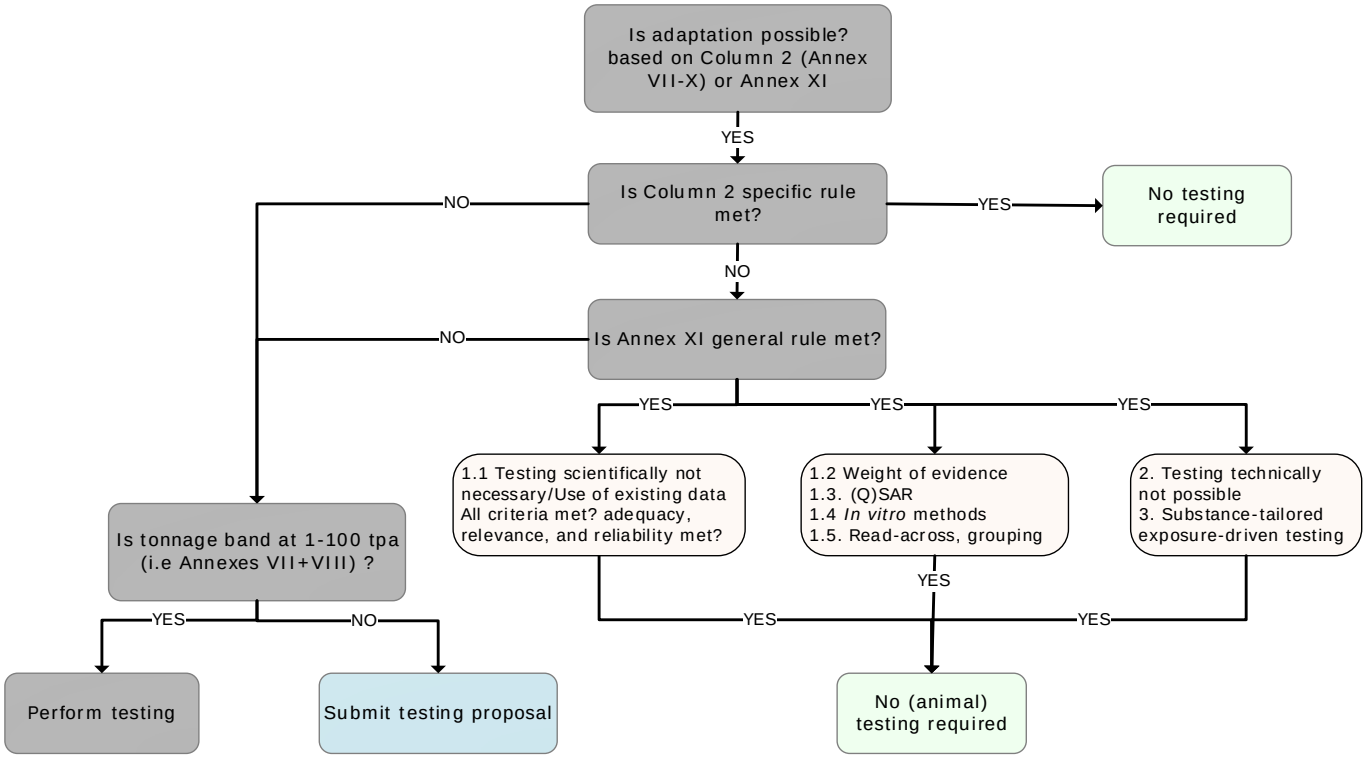
Subsections of Annex XI lay out general rules that may be used:

(i) in section 1, the rules to adapt testing requirements are described and will be further discussed in the next chapters;
(ii) in sections 2 and 3, the general rules for omitting the tests are developed.

When should it be done?

Figure 3 illustrates the decision-making process for using the different waiving/adaptation options.

Figure 3: Decision scheme for waiving/ adapting a standard information requirement



How should it be done?

The four-step process of fulfilling the information requirements is described in Chapter 3 of this practical guide. Note that under REACH, registrants are obligated to provide more than minimal information in relation to each specific endpoint (Step 1). It, in fact, requires the submission of “all” or “any” available information that would be “relevant”. This can serve the

use of waiving argumentation.

For more detailed guidance on the use of adaptations, please consult the *Guidance on information requirements and chemical safety assessment, Chapter R.5*, and in the integrated testing strategies (ITSs) for specific endpoints in R.7 a-c.

You may find more detailed information on the individual options for waiving information, in the chapters below, and in the *Practical Guide on How to use and report (Q)SARs*.

For more information on how to record it in IUCLID, consult Chapter 9.7.2 of the Manual "*How to prepare registration and PPORD dossier*".

General rules of Sections 2 and 3 of Annex XI to REACH

Section 2: Testing is technically not possible

The REACH legislation acknowledges that in some cases, testing for certain endpoints may not be technically possible, and in such cases the test may be waived. For example, testing may not be possible because the substance is not sufficiently soluble in water.

In addition, testing may not be technically possible if the analytical methods available are not sufficiently sensitive to conduct the test for a particular substance. In all such cases, you need to provide a clear justification, and supporting documentation, for why the test is considered to be technically not possible.

Section 3: Substance-tailored exposure-driven testing

The REACH legislation allows "exposure-based waiving" for the tests in Sections 8.6 and 8.7 of Annex VIII and for the tests in Annex IX and X.

To qualify for exposure-based waiving, you need to provide the following:

- exposure scenarios developed for your substance in the chemical safety report;
- adequate and well-documented justification, with supporting documentation that fulfils all conditions listed and is based on thorough and rigorous exposure assessment;
- Demonstration of the strictly controlled conditions (as described in Article 18(4)(a) to (f) apply to the substance).

Specifics for low-risk phase-in substances, manufactured or imported between 1-10 tonnes per year (Annex III to REACH)

If you can demonstrate that your phase-in substance, manufactured or imported between 1-10 tonnes per year, can be considered of "low risk", you may be able to register it providing a reduced set of information, covering only physicochemical properties.

You first need to confirm that the substance does not meet any of the two conditions set in Annex III:

a) there is an indication that the substance could have CMR or PBT/vPvB properties;

b) the substance would likely be classified as hazardous under CLP (for any of the human health and environmental properties), and has dispersive or diffuse uses.

ECHA has published an inventory of substances, which are likely to fulfil the criteria for being hazardous, and therefore likely to require the full set of Annex VII standard information.

The inventory is meant to help you decide whether you may be able to register your substance, manufactured or imported at tonnages between 1-10 tonnes per year, with limited information.

Together with the inventory, ECHA has published a five-step 'checklist' to help you conclude whether you can benefit from the submission of a reduced number of information requirements. In addition, we provide advice on how to use the inventory, including illustrative examples.

In any case, you are still obligated to provide any, and all, available relevant information you have for the substance.

Expertise required

Administrative expertise	If available results can be used directly as input in the registration dossier.
Scientific expertise	If a decision needs to be made on whether or not to perform a test, according to Figure 3. If results of a test are available but there is a need to interpret the results and to conclude on a relevant value for assessment.
Advanced scientific expertise[5]	For any of the approaches proposed under Sections 1.2, 1.3, 1.4 and 1.5 of Annex XI, and to assess whether scientific justification and reliable documentation of such data can be provided, and meeting all conditions. If your substance is on the Annex III inventory and there is a need to provide a justification to possibly overrule the concerns listed in the inventory.

Additional tips

You must document the considerations for applying the Annex III exemption in the IUCLID dossier, section 14. For more details, consult the example cases of Annex III on ECHA's website.

[5] *Guidance on information requirements and chemical safety assessment - Evaluation of available information - Chapter R4*

3.4 Generating new data and submitting testing proposals

The review of all the available information may nevertheless result in concluding that new data needs to be generated to fulfil the information requirements. For information gaps referring to standard information requirements listed in Annexes VII and VIII, you are allowed to generate new information, whereas for information gaps referring to information requirements listed in Annexes IX and X or studies following up certain Annex VIII studies, you need to first prepare and submit a proposal for testing to ECHA in your registration dossier[6].

To avoid any unnecessary animal testing, as a precondition before any new tests are carried out to fulfil the information requirements, you need to first assess all existing and available data (see Chapter 3.1). In practice, this also means that you should have already carefully considered the rules for waiving of Column 2 (see Chapter 3.3) and the general rules for adaptation (see Annex XI to REACH and Chapter 3.3 and 4) before conducting testing on animals.

Pending the availability of results from any new testing, you must also implement the appropriate risk management measures as well as document those you recommend to downstream users.

Need to report your considerations of use of alternatives when you submit a testing proposal

As testing on vertebrate animals should be a last resort, since September 2015 you **must** provide your considerations of alternative methods and why animal testing is necessary.

These considerations should provide meaningful information which addresses each of the adaptation possibilities foreseen under Column 2 of the applicable Annex (IX or X) or under Annex XI. You must provide these considerations in the IUCLID field <*Justification for type of information*> of each endpoint for which testing on vertebrates is proposed, using the available (free-text) template in that field. Note that this information is subject to completeness check and will be disseminated.

You then need to wait for ECHA's decision on your proposal before conducting the test on vertebrate animals. You can find further information on the testing proposal examination and decision making on ECHA's website and in Practical Guide: *How to communicate with ECHA in dossier evaluation*.

[6] In accordance with Articles 10(a)(ix) and 12(1)(d) and (e).

4. Alternatives to avoid animal testing

The various possibilities under this chapter correspond to the Annex XI sections: weight of evidence relates to Section 1.2, (Q)SAR relates to Section 1.3, *in vitro* data relates to Section 1.4, and read across and categories relates to Section 1.5.

4.1 Weight of evidence

What is it?

The weight of evidence approach commonly refers to combining evidence from multiple sources to assess a property under consideration. It can therefore be a useful technique where, for example, each piece of information or test alone is not sufficient to address a standard information requirement but where it may be possible to combine the strengths and weaknesses of the individual studies to reach a conclusion for a particular property.

The term weight of evidence (WoE) is neither a scientifically well-defined term nor an agreed formalised concept characterised by defined tools and procedures[7]. It can, however, be regarded as an evidence-based approach involving an assessment of the relative weights (values) of different pieces of the available information that have been gathered. Application of this concept can be achieved either in an objective way by using a formalised procedure or by using expert judgement. Factors such as the quality of the data, consistency of results, nature and severity of effects, relevance of the information will have an influence on the weight given to the available evidence.

Within the REACH legislation, the WoE approach is a component of the procedure to decide on a substance's property and thus it is an important part of the chemical safety assessment.

The WoE concept was also used in the development of integrated testing strategies. For example, strategies involving a sequence of defined tests to build a weight of evidence have been formalised within the REACH standard information requirements in the case of, for example, skin/eye irritation/corrosion and mutagenicity testing. ECHA's *Guidance on information requirements and chemical safety assessment, Chapter R7a* gives other examples.

Finally, the WoE concept also has a particular application in Annex XI to REACH as an option to meet the information requirements of Annexes VII-X as follows:

"Animal tests can be avoided if there is a weight of evidence which points to the likely properties of a substance. This approach may be applied if there is sufficient information from ***several independent sources*** *leading to the conclusion that a substance has (or has not) a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion […].*

Where sufficient weight of evidence for the presence or absence of a particular dangerous property is available:

- further testing on vertebrate animals for that property shall be omitted,
- further testing not involving vertebrate animals may be omitted.

In all cases adequate and reliable documentation shall be provided."

[7] Weed D (2005): weight of evidence: a review of concepts and methods. Risk Analysis, 25(6): 1545-1557.

It specifically refers to using evidence from *several sources*, where the information from each of the sources individually may be regarded as not sufficient.

The WoE approach by its nature requires the use of scientific judgement, and therefore it is necessary to provide adequate and reliable documentation to justify the use of this approach. The weight of the evidence will not always be sufficient to avoid further testing but may be useful in developing an integrated testing strategy (ITS), since the available evidence can help in the targeting of the subsequent steps and most suitable test.

This chapter details the sources of information that might contribute to a weight of evidence.

When should it be done?

Once existing data has been gathered, the WoE concept provides the opportunity to:

- make use of less reliable information or studies, which may individually, not be adequate to qualify as key studies;
- make a conclusion on a substance property; and thus
- fulfil your information requirements.

It is one way to optimise the use of all available information and different data sources for an endpoint, which can provide sufficient information when used in combination with other studies i.e. to allow a WoE analysis to be made.

It is important to document and explain how the evidence-based approach was used in a reliable, robust and transparent manner. Further information may not be necessary if you provide a rationale to show that the data, combined in a WoE, adequately describes the property under consideration.

How should it be done?

Firstly, the WoE approach involves gathering all available information: the more information the better, and then it requires assessing with expert judgement the gathered information and considering whether a weight of evidence case can be built.

Gather all relevant information

To start building the WoE case, you should gather all existing and relevant information, from all possible sources. Further details on the use, scoring and reporting of existing information are given in Chapter 3.1.

Assessing the overall package to conclude on a property

Cumulative weight: "pooling" of information

There may be several studies available for the same test substance for the same endpoint, which are not deemed to be fully reliable, or to qualify as key studies. However, when used in combination, the study results may indicate an effect at approximately the same concentration and time. In these cases, there could be justification for using all the studies collectively to conclude on a particular endpoint and to satisfy an information requirement.

Examples of studies that are inadequate to qualify as key studies include:

- Problematic tests: where a reasonable estimation of the exposure concentration cannot be determined, then the test result should be considered with caution unless as part of a WoE approach.
- Klimisch 2, 3 & 4 score studies

- Studies conducted according to non-standard guidelines

Example (short-term toxicity to fish)

To address an Annex VIII, Section 9.1.3 endpoint (short-term toxicity to fish), you may have:

- Valid fish toxicity data, only available for a short exposure regime (e.g. 24h);
- Tests with over 96-hour exposure but which cannot be judged as reliable (e.g. because of poor documentation), although they provide information that the main effect occurs within the first 24h. Hence, the 24-hour value might be used;
- Toxicity data for several time points from a 72-hour test; thus, the time-effect curve may allow extrapolation of the 96h value.

When evaluating existing data, it can be expected that the entire study information will not be available to fully assess all of the considerations above. However, the studies may be of good quality and can be considered for use as part of a weight of evidence approach. Please ensure you hold key information to give some confidence that the underlying data is of good quality.

Where such circumstances exist, it is critical to know whether the test was conducted to the standard test guidelines, and the study method should be reported. In addition, key information should also be provided in the technical dossier. These are:

(i) test substance identification;
(ii) sample purity;
(iii) test species; and
(iv) test duration. Further guidance is given in the Guidance on registration.

How to deal with conflicting study results

A WoE approach can be used when several available studies give conflicting results: each study will be rated and provided a weight depending on the test method, quality of the data and the endpoint under consideration. Then the conclusion will be drawn according to the balance of the various weights.

Note that high quality *in vivo* (read-across information) and *in vitro* data would generally carry more weight in the decision than a QSAR or an in-house *in vitro* method.

Expert judgement

Expert judgement is vital in the construction and appraisal of the WoE package, namely when considering the reliability, relevance and adequacy, integrating and comparing different pieces of information and assigning a weight to each piece of data.

The experts providing this scientific judgement must have expertise concerning the relevant endpoints and study methods, as they will need to assess the reliability, relevance, adequacy of the available data and to conclude whether the combined evidence is enough to draw a conclusion about the properties or the potential effects of the substance.

Where test data may not be available or allow a firm conclusion, the use of other information and using expert judgement may allow a conclusion to be drawn.

It is essential that all information used, all steps carried out in the evaluation process and all conclusions drawn are fully documented and scientifically justified in the technical dossier to make the expert judgement transparent and comprehensible.

Report and record the relevant information

To meet the information requirement for an endpoint, you must submit your WoE in the endpoint section of the IUCLID dossier. For each piece of evidence, you should create an individual endpoint study record (ESR), and select "weight of evidence" in the field <Adequacy of study>.

You should then provide the information in the form of a robust study summary: you need to fill in all the relevant information under the ESR headings "Administrative data" (such as "Type of information" and "Reliability"), "Data source", "Materials and methods" and "Results and discussion" (see the case studies at the end of the chapter).

Every ESR submitted as part of WoE approach will be subject to a completeness check during the registration process, as are the ESRs submitted as key studies.

For more information on preparing registration dossiers in the IUCLID format, and on the completeness check, consult the Manual on *"How to prepare registration and PPORD dossiers"*: Chapter 9.7.4 for examples of completing endpoint study records and Annex 2.

Recommendations

1 Prepare an endpoint summary, stemming from the various ESRs, where the findings for the endpoint are summarised as well as the rationale for the conclusion you reached;

2 Provide enough data for each piece of the weight of evidence to enable ECHA to evaluate the overall evidence and to demonstrate that the combined information allows for a rational judgement to be made on the physicochemical, ecotoxicological and toxicological intrinsic properties of a substance;

3 Clearly document and report your scientific considerations of the pieces of evidence and overall judgement to enable ECHA to evaluate the overall evidence in an unbiased way;

4 All endpoint study records that are part of a WoE approach **must be flagged** as such in the field <Adequacy of study>;

5 Weight of evidence must not be flagged if the registrant intends to waive a study based on Column 2 of the REACH Annexes VII-X;

6 Provide **robust study summaries** for each study used as part of a WoE approach;

7 Always consider the quality of the available data, the consistency of the results, the severity and the type of effects of concern and the relevance of the available data for the property.

Expertise required

As previously described, scientific expertise is required, per endpoint, except where available data is entered in IUCLID. Every case will be different.

Administrative expertise	If available results of a test, including with the relevant Klimisch scores, can be used directly as input in the registration dossier.
Scientific expertise	If available results require an interpretation or to be provided with Klimisch scores to conclude on a relevant value for further assessment.
Advanced scientific	If multiple sources of evidence, either from experimental data

expertise	or not, can be used as alternatives to standard testing; building the weight of evidence approach and ensuring the appropriate and reliable documentation; assessing the conditions of Annex XI, Section 1.2.

Additional tips

1 The dossier must always contain a well-documented and valid justification for adapting the standard information requirements, which is based on scientific argumentation, and documentation of underlying evidence.

2 ECHA only accepts a WoE approach if it is substantiated in IUCLID by several ESRs along with appropriate documentation on the various sources of evidence; you need to use the correct flags in the ESRs and to have an endpoint summary which overarches the other ESRs related to each of independent piece of evidence.

3 ECHA has observed that registrants have made inappropriate or inadequate use of the WoE approach in trying to use several sources of less adequate existing information.
As an exception, when substantial argumentation can be used to justify not carrying out a test based on lack of exposure, you should not flag the endpoint study record as 'weight of evidence' but should instead indicate a data waiving, selecting the reason 'exposure considerations'. You should then provide adequate quantitative justification based on the exposure scenarios developed in the chemical safety report (CSR);

4 Advanced methodologies such as toxicogenomics can also inform on the risk assessment and assist decision making for designing efficient and effective testing strategies as well as providing the mechanistic basis with which to address the mode of action, biological relevance of the effects observed in *in vivo* studies and human relevance.

Case studies

Case study 1: adequate application of a WoE approach, for the endpoint 'Water solubility', based on two lines of evidence: read-across and QSAR prediction.

In such a case, two main endpoint study records (ESRs) accompanying the ESR for the source substance need to be provided. The accompanying ESR provides the basis only for a read-across approach (not for the weight of evidence).

The first ESR (a) provides the basis for the read-across approach. This is an experimental result for a structurally-related substance (analogue, source for read-across), the field <Type of information> is set to "experimental study", the field <Adequacy of study> is filled with "key study", the robust study summary box is ticked. All the relevant fields for a robust study summary (RSS) are filled in, including the registrant's interpretation and conclusion. The registrant may also attach a supporting document or report in the ESR.

The second ESR (b) is the read-across **target** (outcome of read-across) and serves as a read-across from supporting substance (structural analogue or surrogate); the field <Type of information> is set to "read-across from supporting substance (structural analogue or surrogate)", the field <Adequacy of study> is filled with "weight of evidence". A cross-reference is made to the ESR (a) representing the source study in the field "Cross-reference". In the field <Justification for type of information>, a justification for the read-across approach is provided. The registrant may also attach a supporting document or report in the ESR.

The third ESR (c) is for a (Q)SAR prediction, where the field <Type of information> is set to "(Q)SAR", the field <Adequacy of study> is filled with "weight of evidence" and all the fields required for a robust study summary were filled in. In the fields <Justification for type of information> and <Attached justification>, the documentation behind the QSAR prediction is

provided.

An endpoint summary is created to cover the main findings of the individual ESRs. In addition, the registrant further documents how they ascertained the property for the substance from the WoE approach.

For more information, consult Chapter 9.7.2 of the *Manual "How to prepare registration and PPORD dossiers"*.

Case study 2: inadequate application of a weight of evidence approach

Only one ESR marked as "weight of evidence" (in the field <Adequacy of study>) is provided, and presents a Klimisch-4 experimental study.

This is not sufficient to make an evaluation or to meet the information requirement. It is therefore important that the registrant builds a stronger package of evidence, drawing on additional sources of information and that they document the pieces of evidence and rationale on conclusions for the endpoint.

4.2 (Q)SAR

What is it?

Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) models – collectively referred to as (Q)SARs – are theoretical, computerised models that can be used to predict in a quantitative or qualitative manner the physicochemical, biological (e.g. an (eco)toxicological endpoint) and environmental fate properties of substances, from the knowledge of their chemical structure. These models are included in free and commercial software packages.

The use of (Q)SARs (also named *in silico* approach) may allow you to avoid unnecessary testing, including animal testing, if the information obtained is sufficient to fulfil the information requirements. However, these predictions can be considered valid and be used only when certain conditions are met.

The approach of using (Q)SAR models seeks to predict the intrinsic properties of chemicals by using various databases and theoretical models, instead of conducting tests. Based on knowledge of chemical structure, QSARs quantitatively relate characteristics of the chemical to a measure of a particular activity, while SARs allow qualitative conclusions about the presence or absence of a property of a substance, based on a structural feature of the substance.

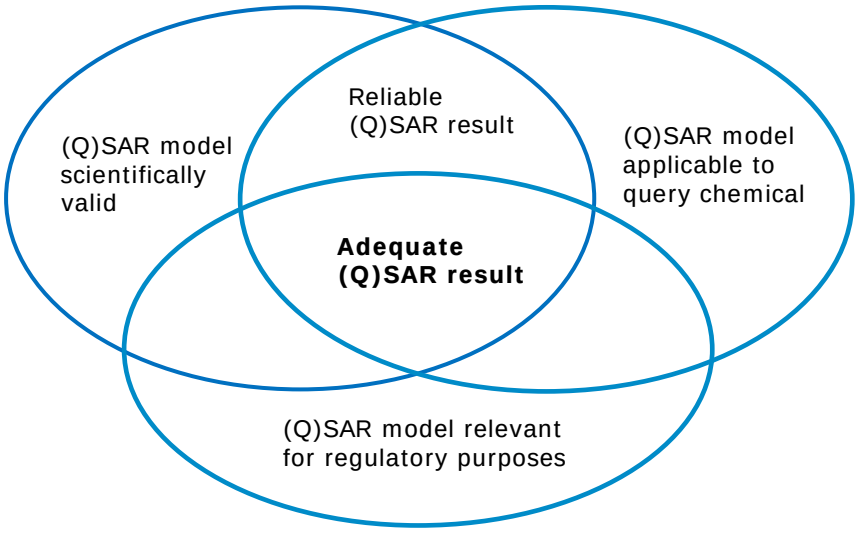
As with any other form of data, you need to provide sufficient documentation to allow for an independent evaluation of the results. Further details on (Q)SAR models are available in the Practical Guide on “How to use and report (Q)SARs”.

When should it be done?

(Q)SAR predictions can be used instead of testing, as an acceptable adaptation, only when adequate (Q)SAR results are available, i.e. the conditions set out in REACH Annex XI, Section 1.3, are met:

(i) the substance has to fall within the model’s applicability domain;
(ii) the results must be obtained using a scientifically valid model;
(iii) the results must be sufficient for the purposes of classification and labelling, and risk assessment purposes; and
(iv) the information should be well documented.

Figure 4: Scheme on how to identify an adequate (Q)SAR result.



The scientific validity of the model is evaluated according to the following principles:

(i) a defined endpoint;
(ii) an unambiguous algorithm;
(iii) a defined domain of applicability;
(iv) appropriate measures of goodness-of-fit, robustness and predictivity; and
(v) a mechanistic interpretation, if possible.

Some simple properties and endpoints can be reliably predicted by using (Q)SAR models, and if the substances fall within the applicability domain of the model, while for higher-tier endpoints (Q)SARs can only give preliminary indications on the type of toxicity the substance may exhibit.

Experience and a thorough understanding of QSARs are needed to verify the reliability and adequacy of the predictions.

How should it be done?

In general, you should use (Q)SAR results as part of a weight of evidence approach (see Chapter 4.1 of this guide) or an integrated testing strategy.

ECHA's experience of using adaptations to address standard informational requirements reveals that there are no simple (Q)SAR solutions for complex health endpoints such as repeated dose toxicity, developmental and reproductive toxicity in general.

When using (Q)SARs, you should run all the available (Q)SAR models for the endpoint. The available models should be independent (different in terms of prediction formalism and underlying data).

You need to verify that your (target) substance falls within the applicability domain (AD) of the model. In practice, you need to check the following elements:

(i) descriptor domain;
(ii) structural domain, mechanistic and metabolic domains, if possible.

Having close structural analogues in the training set of the model increases the reliability of the prediction, especially if the analogues are predicted correctly, or in the acceptable margin of error. Analogues can be searched from the model training and/or test set, as well as in available databases (e.g. like in the OECD QSAR Toolbox database).

Finally, you need to submit the proper documentation to support your justification:

(i) (Q)SAR prediction reporting format (QPRF) to document the prediction; and
(ii) (Q)SAR model reporting format (QMRF) to document the model.

While the QMRF is a general description of the model and is usually provided by the developer, the QPRF is prediction-specific and needs to be prepared for each prediction.

Further details and guidance on the use of (Q)SAR data are available in the *Practical Guide on "How to use and report (Q)SARs"* and the *Guidance on information requirements and chemical safety assessment, Chapter R.6*.

Expertise required

Advanced scientific expertise To understand the computational models (Q)SARs as the use of, justification for, and documentation of such data is subject to very specific rules; assessing the conditions of Annex XI, Section 1.3.

Additional tips

1 Disregard the predictions that fulfil only some conditions specified in REACH Annex XI, Section 1.3 or explain the reason for providing these predictions. The closer to a regulatory threshold the predicted result is, the more accurate the prediction needs to be.

2 The QMRF describing the scientific validity of the model may be attached to the endpoint study record, while the QPRF for the specific prediction should always be attached or equivalent information should be provided in the pre-fillable field <Justification for type of information> in IUCLID.

3The use of the OECD QSAR Toolbox does not replace the need to prepare a QPRF to describe the scientific reasoning or to provide supporting evidence for the prediction.

4 Consider the specific chemistry of your substance to decide whether the substance falls in the applicability domain of the model or if it can be difficult to predict e.g. information on reactivity or specific modes of action can highlight structures where excess toxicity would be expected, and predictions may be potentially less accurate.

4.3 *In vitro* data

What is it?

A test performed *in vitro* (Latin: in glass) is performed in a controlled environment, such as a test tube or petri dish, outside of a living organism. In contrast, a test performed *in vivo* (Latin: in the living) is one using a living organism, e.g. a vertebrate animal.

Results obtained from suitable *in vitro* methods may indicate the presence of a certain property or may be important in relation to understanding the mode of action of the substance. In this context, "suitable" means sufficiently well developed according to internationally agreed test development criteria (e.g. the European Centre for the Validation of Alternative Methods (ECVAM) pre-validation criteria). Validation is the process by which the reliability and relevance of a procedure is established for a specific purpose.

Through the promotion of alternative methods, several *in vitro* test methods have undergone international validation and been accepted for regulatory use.

When used as an adaptation, and if the results of an *in vitro* test indicate the absence of an intrinsic property, the standard test may still need to be performed to confirm the absence of the property. Exceptions include those *in vitro* tests for which negative results may be accepted, when used as part of an integrated approach. For example, when *in vitro* tests are already accepted as standard information requirements (e.g. for skin corrosion/irritation and serious eye damage/eye irritation endpoints) or they are essential steps in a standard integrated testing strategy (e.g. in the case of mutagenicity).

In all cases, the data generated using *in vitro* methods needs to be obtained using a scientifically valid method, and to be adequate for the purposes of classification and labelling and/or risk assessment. As with any other form of data, you need to provide sufficient documentation to allow the results to be independently evaluated.

In the EU, ECVAM is responsible for coordinating the scientific validation of new alternative testing methods. There are five main steps identified in the evolution of new test methods, which include test development, pre-validation phase, validation phase, independent assessment and finally progression towards regulatory acceptance.

The pre-validation process is essential to ensure that any method included in a formal validation study adequately fulfils the criteria defined for inclusion in such a study. Pre-validation and validation principles and criteria for how validation studies of new or updated test methods should be performed are described in *the Guidance on information requirements and chemical safety assessment, Chapter R.4* and are adopted from the OECD Guidance Document 34. You can find more detailed information on the use of such *in vitro* methods, in the Guidance document and at http://ecvam.jrc.it/.

Categories of in vitro methods and data

There are three categories of *in vitro* methods and data that can be used for the purpose of registering substances under the REACH Regulation.

(i) Validated *in vitro* methods

Validated *in vitro* methods, once scientifically agreed according to internationally agreed validation principles, are usually listed in the Test Methods Regulation and/or in the OECD Test Guidelines, and can fully or partly replace an *in vivo* test depending on the purpose for which the method was validated and adopted.

Some *in vitro* test methods are among the standard information required at different tonnage levels (e.g. *in vitro* assays for skin and eye irritation, skin sensitisation, *in vitro* assays to assess mutagenicity). These are validated methods that have been proven to be adequate and suitable for providing information for the purpose of classification and labelling and/or risk assessment.

(ii) Pre-validated *in vitro* methods

In vitro tests that meet the internationally agreed pre-validation criteria are also considered suitable for use under REACH when the results from these tests indicate a certain dangerous property. However, if the results from pre-validated methods do not indicate a dangerous property (negative results) these have to be confirmed with the relevant test specified in Annexes VII-X for the corresponding endpoint (Annex XI, Section 1.4). Alternatively, the results can be part of a weight of evidence (WoE) approach.

When data from pre-validated *in vitro* methods are used, the ECVAM criteria for entering the pre-validation phase including evidence of the reproducibility of the method, its mechanistic relevance and predictive capacity need to be provided in the registration dossier.

(iii) Non pre-validated *in vitro* methods

In addition, pre-validated methods and other *in vitro* data (non pre-validated) can be used to gather information to provide additional data for the evaluation and interpretation of *in vivo* or *in vitro* data, as part of the mechanism of action (e.g. kinetic *in vitro* data, toxicogenomics, metabolomics), and for supporting the adaptation of the standard testing regime, as specified in Annex XI (use of existing data, read-across and grouping of chemicals, and/or weight of evidence).

You always need to define the purpose of use of such methods in a clear and well–documented scientific justification. Where applicable (e.g. pre-validated methods used as supportive evidence) the criteria for suitability have to be provided.

When should it be used?

According to Article 13(1) and (3), *in vitro* tests are suitable to generate information on intrinsic properties before considering *in vivo* animal testing. In addition, Annex XI, Section 1.2 mentions that *"newly developed test methods, not yet included in the test methods referred to in Article 13(3)",* and which may be still in the pre-validated stage, could be considered within a WoE approach.

Amendments to the REACH annexes have been implemented in favour of alternative test methods, including *in vitro* methods. They enter into force in two steps: first for skin and eye irritation and acute dermal toxicity, and then for skin sensitisation.

The new requirements for skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation make non-animal testing the default, whereas another change provides additional adaptation possibilities to address acute dermal toxicity. In most cases, the information needed for the classification or risk assessment of a substance will now be obtained through *in vitro* studies only.

In vitro data can therefore be used either to fully or partly replace information requirements that would otherwise have to be generated with *in vivo* data. However *in vitro* data, including those generated by methods not meeting internationally agreed validation criteria (adequacy and suitability) for a specific endpoint, also have to be considered and submitted in the registration dossier as part of gathering all available information and used in a WoE approach (Annex XI, Section 1.2) or supporting the grouping of substances (Annex XI, Section 1.5).

How should it be used?

Assessing and reporting adequacy and suitability

Before you submit a validated *in vitro* test method in your registration dossier, you need to assess the quality criteria for the corresponding endpoint and you need to report them in the relevant IUCLID endpoint study record (ESR). As long as the method is listed in the EU Test Method Regulation or in the OECD Test Guidelines, the adequacy for use for a specific endpoint was assessed at international level and the method can be used to fully or partly replace animal testing.

You must take into account any limitations described in the test method protocol or in the technical guidance documents. For example, some *in vitro* methods are adequate only for the prediction of positive results (indication of a dangerous property) but not for negative results.

You should also check that your substance is suitable to be tested using the *in vitro* model you choose, as there may be limitations of the applicability domain of the test.

If you wish to use pre-validated *in vitro* methods to fulfil REACH information requirements, you must fulfil the conditions specified in Annex XI, Section 1.4, and assess the suitability of the method against the ECVAM criteria before reporting the results in the IUCLID dossier. If you want to use any other *in vitro* methods as part of a WoE approach, you first need to assess the quality of the method and the quality standards (reproducibility of test results) before including your considerations in the IUCLID dossier as part of the ESR.

Use for the purpose of classification and labelling and/or risk assessment

Only validated and pre-validated *in vitro* methods can be used under specific conditions for the purpose of classification and labelling and/or risk assessment. If you use a validated *in vitro* method, listed in Annexes VII-X, or a pre-validated test method indicating the dangerous properties of a substance, the results can be considered adequate for classification and labelling and/or risk assessment. Other *in vitro* data can be used only as part of the WoE approach to support decision-making.

Regarding serious eye damage/eye irritation (Annex VII, Section 8.2), you need to gather or generate information for the classification and risk assessment of a substance through *in vitro* studies. In some cases, combinations of *in vitro* studies can be used and will be sufficient. In other cases where conclusions on C&L cannot be drawn, *in vivo* studies may still be required, to meet the information requirements according to Annex VIII, Section 8.2, column 2. The same principle applies to the property “skin corrosion/irritation”.

Regarding skin sensitisation (Annex VII, Section 8.3) and because of changes of the REACH annexes, if no conclusion can be drawn from these tests **or** if the available *in vitro/in chemico* test methods are not applicable for the substance (see Scenario 2), you may then be allowed to perform the *in vivo* test (Annex VII, Section 8.3.2).

Recommendations

1 Data generated from *in vitro* (validated and pre-validated) test methods can be used under REACH if the information for the hazard endpoint is sufficient for the purpose of classification and labelling and/or risk assessment.

2 Advanced *in vitro* technologies may provide valuable information on the mode of action of the substance and can be part of a read-across and category justification.

3 *In vitro* data produced from (non-)pre-validated methods can only be used as supportive information (e.g. as part of a WoE justification).

4 You should always report the results in a detailed, clear manner, including the test conditions

and the interpretation of the usefulness of the results in your registration dossier. This applies if the study is used as a key study or as part of a WoE approach.

5 Limitations in the method should be clearly communicated; for example *in vitro* test methods may not replicate all of the metabolic processes that may be relevant to chemical toxicity that occur *in vivo*.

6 The conditions set out in the REACH Regulation Annex XI, Section 1.4 must be met.

Expertise required

Administrative expertise	If available results of a test, including its relevant Klimisch scores, can be used directly as input in the registration dossier.
Scientific expertise	If available results require an interpretation or to be provided with Klimisch scores, to conclude on a relevant value for further assessment.
Advanced scientific expertise	If multiple sources of evidence, either from experimental data or not, can be used as alternatives to standard testing; building the weight of evidence approach and ensuring the appropriate and reliable documentation; assessing the conditions of Annex XI, Section 1.4.

Additional tips

How to report in vitro methods in IUCLID depending on their validation status[8]

When you are using results from a validated *in vitro* method in your registration dossier to fulfil REACH requirements, you need to provide the robust study summary or study summary in your IUCLID registration dossier. You will need to provide a sufficient description of the test conditions, results and interpretation for the purposes of decision making regarding classification and labelling and/or risk assessment.

If you are submitting the results of a pre-validated *in vitro* method, as a key study with the purpose of fulfilling data requirements for a specific endpoint, the relevance of the method has to be made clear. In addition to the requirements on RSS, you need to include documentation demonstrating that the method meets the criteria for suitability assessment according to the ECVAM criteria in the registration dossier, to assess the suitability of the method and its potential acceptance for classification and labelling and/or risk assessment.

Remember that if the results from such methods do not indicate certain dangerous properties for the specific endpoint addressed, you need to carry out the required test to confirm the negative results, unless testing can be waived according to other specific and general rules for adaptation of the standard information requirement.

If you submit the results from a pre-validated or a non-pre-validated *in vitro* method, as supportive studies, or as part of a weight of evidence approach or as disregarded studies, you need to indicate it clearly together with providing the appropriate and well-documented justification in your registration dossier, using the relevant IUCLID fields in the ESR.

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[8] *Practical Guide on "How to report robust study summaries" (RSS)*, the *Manual on "How to prepare registration and PPORD dossiers"* and the IUCLID Help system, which is accessed by pressing F1 while inside the IUCLID application.

If *in vitro* information is used within the weight of evidence context, details on the method need to be provided in the IUCLID format for RSSs. The relevance of the findings of the studies in relation to conclusions drawn from the overall data set also has to be documented in detail. In addition, if some studies that are flawed but indicate critical results, you also need to prepare RSSs highlighting the weaknesses of the studies.

Such studies can be flagged as 'disregarded due to major methodological deficiencies' in the field <Adequacy of study> in IUCLID.

How to report in IUCLID the use of in vitro data to fulfil a standard information requirement

When you report any results in your IUCLID registration dossier, you need to provide a justification for adapting the standard testing regime.

Scenario 1: You have *in vitro* information when *in vitro* results have become the standard requirement

Regarding skin sensitisation, *in chemico/in vitro* methods are expected to become the standard information requirement by the end of 2016 (Annex VII, Section 8.3.1), and it may then be possible to determine the skin sensitisation potential of a substance within a testing strategy by using a battery of *in chemico/in vitro* methods.

Information addressing three key events of skin sensitisation has to be provided unless information from less allows the substance to already be correctly classified i.e. whether the substance is a skin sensitiser or not. If the substance is a skin sensitiser, the skin sensitisation potency should be assessed and differentiation between sub-categories 1A and 1B is required.

In your registration dossier, you will need to report the *in chemico/in vitro* results as a weight-of-evidence with the appropriate justification.

You need to create a separate ESR for each of the *in chemico/in vitro* methods you have performed, to derive the final conclusion on classification for your substance: you may have to create one to three separate ESRs, if you are able to classify after the first or second test.

Then, for each piece of available evidence, you need to fill in for each ESR, the <Adequacy of study> field by choosing in the pick-list, “weight of evidence” or “supporting study” (see Chapter 4.1). Remember that submitting only supporting studies for an endpoint is not sufficient.

For more information, consult Chapter 9.7.2 of the Manual “How to prepare registration and PPORD dossiers”.

Technical completeness check (TCC)

All studies marked as “Key study” and “Weight of evidence” are subject to the completeness check, which is the required step to successfully submit your registration dossier.

Scenario 2: you have *in vivo* information when *in vitro* results have become the requirement

Because of changes to the REACH annexes, some *in vivo* tests are no longer the default information requirement for some endpoints (for example, for eye irritation or skin irritation).

If *in vivo* tests alone should be available (for example, because the substance does not lie within the applicability domain of the *in vitro* test), you can submit the *in vivo* study, while having to provide a waiving statement for the *in vitro* requirement (i.e. adaptation justification). Note that a standard waiving phrase is available in IUCLID.

In the first (*in vitro)* ESR, you need to indicate that you are waiving the *in vitro* testing, according to the (specific or general) rule you wish to apply, by choosing the correct entry in the <Justification for data waiving> pick-list field, because you already have *in vivo* information.

Administrative data	
Endpoint	Skin irritation: *in vitro/ex vivo*
Data waiving	Study scientifically not necessary/other information available
Justification for data waiving	Pick the correct justification: An *in vitro* skin irritation study does not need to be conducted because adequate data from an *in vivo* skin irritation study are available
Cross-reference	<Link to Section 7.3.1 endpoint study record (key study or weight of evidence records) for Skin irritation: *in vivo*.>

Note: You may also justify that the *in vitro* methods are not suitable for your substance, if that is indeed the case.

In the second (*in vivo*) ESR, you then submit the full robust study report information by filling all appropriate fields.

Regarding, skin or eye irritation (Annex VII, Sections 8.1 and 8.2), if you cannot draw conclusions on classification and labelling, *in vivo* studies may still be required. You will need to submit an ESR for an *in vivo* study, while also providing the results of the *in vitro* studies (with justification <cannot be used for classification>).

4.4 Read-across and categories

What is it?

Read-across in REACH is a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s) (source substance(s)). To cover the complexity of each endpoint, it needs to be clear how the read-across addresses the endpoint or property under consideration.

Substances with physicochemical, toxicological and ecotoxicological properties that are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances.The term "analogue approach" is used when the read-across approach is employed between a small number of structurally-similar substances. As the number of substances is small, trends may not be apparent. As a result of structural similarity, a given toxicological property of one substance (the source) is used to predict the same property of another substance (the target) with the purpose to fulfil a REACH information requirement.

Therefore, it is usually important to have at least one acceptable study of good quality for the endpoint or property under consideration. If several analogues with suitable data are available, a worst-case approach for predictive purposes can be used. In that case, the assessment of the read-across is repeated for each pair of source and target substances such that the worst case is justified.

The "category approach" is used when read-across is employed between several substances that are grouped together based on defined structural similarity and allowable differences between the substances. Because of the structural similarity, the results will be either similar, or follow a regular pattern. The basis for a prediction within the group for the target substance must be explicit (e.g. "worst case", or trend analysis).

Note that under REACH, structural similarity alone is not considered sufficient to justify the possibility to predict property(ies) of the target substance by read-across. The differences in structure should also be explained, i.e. why structural differences, or variations within the group, are not considered to affect the property being predicted.

Use of the Read-Across Assessment Framework (RAAF) may help you assess and, where necessary, improve your read-across. ECHA developed the RAAF based on the most frequently encountered types of read-across approach. These are formulated as scenarios. Each scenario is characterised by a number of scientific considerations, which are crucial to assessing read-across. These are called assessment elements, which include a logical order of questions and possible outcomes (called assessment options) and examples.

Answering these questions helps to determine the level of confidence, and overall acceptability of the read-across approach. The RAAF was developed for mono-constituent substances and human health toxicology. However, the principles it contains may also apply by analogy to environmental endpoints and multi-constituent and UVCB substances.

Similarity of substances

You should establish the basis for grouping the chemicals (in terms of their similarity) using the rules specified in Annex XI, Section 1.5, and further elaborated in the REACH *Guidance on information requirements and chemical safety assessment, Chapter R.6: (Q)SARs and grouping of chemicals.*

The definition of the category approach does not exclude the analogue approach, where in the simplest form it includes one source and one target substance. However, the category justification is considered stronger when more analogues are gathered, and when there are enough bridging data across the members to indicate they are similar enough, or show a

consistent pattern of (non-)toxicity.

The similarities may be due to a number of factors:

(i) common functional group;
(ii) common precursor or breakdown products;
(iii) constant pattern in changing potency; and
(iv) common constituents or chemical class.

These “similarity” rules may be used individually. However, if the category (and similarity) is justified based on more than one basis, for example, only chain length as “allowed” difference and common metabolic pathway, there could be more confidence in the category.

The hypothesis should take into account both routes of exposure and duration of effects.

When should it be used?

When you identify a data gap in your dataset and there are existing tests on analogue substances, you need to consider if you can use read-across to predict the intrinsic properties of your registered substance, based on the “similarity” rules. Analogue substances may also be identified from international assessments (e.g. OECD HPV category approaches) or through use of expert tools, such as the OECD QSAR Toolbox.

If it appears that a potential grouping and read across approach needs to be confirmed or needs strengthening, you may consider conducting or proposing tests to support the category.

How should it be used?

To develop a grouping and read-across approach, you should follow the steps described in the Guidance on information requirements and chemical safety assessment, Chapter R.6: (Q)SARs and grouping of chemicals. Also, you could consider the OECD Guidance on grouping of substances.

How should I characterise the grouping and read-across of substances?

You need to assess the structural similarity of the target and the source substances and the impact of the structural differences between the substances on the endpoints under consideration.

Toxicokinetic information on the substances under consideration, including information on the metabolic fate, can considerably strengthen the robustness of a read-across hypothesis. A clear understanding of the physico-chemical profile of the source and target substances helps to build a read-across case.

A grouping/category definition should document the chemical similarities and trends in properties and/or activities that link the category members with each other. You have to know the boundaries (i.e. applicability domain) and the structural relationship between the category members and define clear criteria for category membership. You should describe all the source and target substances, as comprehensively as possible, including identifiers, purity/impurity profiles and their impact on the endpoints under consideration.

The justification you provide should scientifically explain why the read-across is possible. If the read-across does not contain sufficient, relevant and reliable information on the source and target substances to substantiate the read across hypothesis, it may be necessary to perform or propose further testing to strengthen the justification for read-across. The justification should also address the structural differences between the substances to demonstrate that the differences allowed do not significantly alter the predicted toxicity.

Finally, you should construct a matrix of available data organised in a suitable order, and which should reflect any trends or progression seen within the group. The matrix should indicate whether data are available and whether there are reliable key study results.

Consult the ECHA web pages on Grouping of substances and read-across, presenting the RAAF, an example and related documents. Consider using the RAAF document to assess your read-across.

The OECD QSAR Toolbox can be used to evaluate the category consistency using a number of profilers (IT-coded knowledge, usually in a form of decision-tree). These include:

- pre-defined categorisation of substances (e.g. as defined by the US EPA, or as in OECD category documents);
- empirical (structural) profilers such as organic functional groups);
- structural similarity, endpoint-specific (e.g. for skin and eye irritation/corrosion, for *in vitro* mutagenicity, etc.); and
- mechanistic (e.g. DNA binding protein binding) and toxicological profilers.

Is the read-across prediction adequate for the purpose of classification and labelling and/or risk assessment?

A read-across prediction should be adequate for the purpose of classification and labelling and/or risk assessment. For example, it should not be prone to bias in the selection of source substances or source studies e.g. such that the hazard is under-estimated. The adequacy of the prediction for the purpose of classification and labelling and/or risk assessment under REACH may differ from that needed in other contexts e.g. product development or hazard ranking purposes. In such a case, additional information might be needed to confirm that the generated prediction is adequate in a regulatory context. It also needs to be clear how the prediction addresses each endpoint under consideration due to the different complexities (e.g. key parameters, biological targets) of each endpoint. It may also need to be considered whether the prediction is adequate to allow a conclusion to be drawn according to criteria used for classification purposes e.g. does the prediction address the types of effects and the dose response relationship. In addition, other hazardous properties of a substance partially/not covered by the standard information requirements (e.g. immunotoxicity) may also be relevant to understanding the hazards and risks a substance may present.

When is a grouping and read-across approach properly documented?

It is essential that the justification of the read-across is clearly presented. A read-across should include satisfactory substance identification of all the source and target substances, including constituents, and purity/impurity profiles. The documentation should also contain a detailed description of the hypothesis for the grouping and read-across, including toxicokinetic considerations when used for toxicological endpoints. The read-across justification should include a comparison of the experimental data for the source and target substances and a clear data matrix, highlighting any trends within data. It is important to document a read-across well to allow appropriate evaluation by an assessor.

As well as good documentation, the robustness of a category or read-across from an analogue will depend on the validity of the read across hypothesis and its scientific basis, as well as on the evidence presented.

Guidance of documentation is described in the Guidance on information requirements and chemical safety assessment, Chapter R.6: (Q)SARs and grouping of chemicals.

Substance characterisation

It is critical that you define well the chemical structures and purity profiles of all substances used in the read-across approach, since differences in impurities or stereochemistry can affect the activity and chemical properties. The detailed description of the composition of the source and target substances allows better use of available data. The *Guidance for identification and naming of substances under REACH and CLP* is recommended for all substances used in the read-across. UVCB substances should also be clearly characterised.

How can in vitro data be used in read-across and category building?

Data generated with *in vitro* tests can be used as bridging material between the sources and the target substances, if relevant. *In vitro* or *ex vivo* data can clarify mechanistic considerations (toxicodynamic similarity) and increase the robustness of the read-across hypothesis, in the context of common metabolic products from similar substances, or ADME in general (toxicokinetic similarity).

In addition, *in vitro* data can also be used to demonstrate the biological value of the "mechanistic terminology" used in (Q)SAR models e.g. assisting in defining the applicability domain of a group of substances.

Expertise required

Advanced scientific expertise	If experimental data from one or more analogue substances (read-across/grouping) can be used as alternatives to standard testing; building the read-across/ category approach and ensuring the appropriate and reliable documentation; assessing the conditions of Annex XI, Section 1.5. Use of, justification for, and documentation of such data is subject to very specific rules.

Additional tips

1 You need to substantiate all claims with supporting data. Factual evidence must always be available in the registration dossier, as RSS, individually submitted in an endpoint study record. Hence, a simple reference to other assessments (e.g. in other registration dossiers or other websites or performed under other legislative frameworks) will not be accepted by ECHA. Reports or other supporting information can be attached to the dossier

2 An acceptable read-across justification is normally based on multiple lines of evidence. Different routes of exposure and forms of the substance should also be taken into account. A consideration of information from studies on toxicokinetics may improve the robustness of the read-across hypothesis.

3 The documentation must detail which hazard endpoints are covered by the read-across, and the source chemical used for the read-across must be identified. It is also important that the reliability scores reflect the *assumptions* of similarity. Thus, a Klimisch score of 1 (reliable without restrictions) should normally not be used for results derived from read-across.

4 A comparison of experimental data for hazard endpoints for all category members (also presented in a tabular data matrix) is recommended, ideally highlighting the trends within the category.

5 In IUCLID, you need to specify in each endpoint study record (ESR), whether the identity of the test substance differs from the one defined in section 1 of the dossier (i.e. registered

substance). Furthermore, instructions on how to report read-across in IUCLID are given in the *Manual "How to prepare registration and PPORD dossiers"*.

6 Reading across to information not yet generated (e.g. following the submission of a testing proposal) on an analogue substance is not a valid adaptation. In this case, you indicate that an experimental study is planned and reference the analogue substance for which the testing is proposed.

7 Where substances have been accepted as members of categories under other regulatory programmes (for example, OECD HPV categories), you should refer to them in the dossier. You must nevertheless include all available information (including information which became available after assessment in the other regulatory programme) and reassess the validity of the category according to the REACH information requirements.

Useful Links

Tracking system for alternative test methods review (TSAR) from DG JRC:

TSAR is a tool that provides a transparent view on the status of alternative test methods as they progress from purely scientific protocols submitted for pre-validation to being actively used in a regulatory context.

European Centre for the Validation of Alternative Methods (ECVAM)

OECD: Organisation providing Testing Guidelines to assess chemicals

EC Test Methods Regulation (Council Regulation (EC) No 440/2008)

Further references from ECHA website

How to prepare registration and PPORD dossiers
Practical Guide on how to use and report QSARs
Practical Guide for SME managers and REACH coordinators
Guidance on Registration
Guidance on Data-sharing
Grouping of substances and read-across, including the RAAF
ECHA Webinars on how to use *in vitro* data, read-across, … (2012, 2013, 2014, 2016)

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