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Non-testing in REACH?

ABSTRACT

The REACH regulation which entered into force in June 2007 foresees a large number of tests to be performed in order to register substances. REACH offers the opportunity to apply intelligent testing or non-testing strategies, using alternatives for the tests required for the different tonnage bands. Read-across is a non-testing strategy, which is closely linked to grouping/categorization and (Q)SARs. When applying read-across in an intelligent way, it may not be necessary to develop (and validate) QSARs. In most cases SAR will be sufficient to generate data that allow classification and can be used in risk assessment. Application of read-across is attractive both from the ethical (vertebrate testing) and financial point of view.

The REACH regulation is an EU legislation, which foresees Registration, Evaluation, Authorisation and restriction of Chemicals. The regulation was developed in order to "ensure a high level of protection of human health and the environment as well as the free movement of substances, on their own, in preparations and in articles, while enhancing competitiveness and innovation" (article 1, Regulation (EC) No 1907/2006).

On 13 December 2006 the European Parliament adopted a compromise package on REACH as agreed with the Council on 30 November. The Council adopted the final REACH text at the Environment Council on 18 December. The regulation was published in the Official Journal of the European Union on 30 December 2006 (Regulation (EC) 1907/2006). REACH entered into force on 1 June 2007. The regulation replaces the Dangerous Substances

Directive (Directive 67/548/EEC), the Dangerous Preparations Directive (Directive 1999/45/EC), the Existing Substances Regulation (Council Regulation 793/93/EEC) and the Marketing and Use Directive (Directive 76/769/EEC). This implies that the requirements for new and existing chemicals will be similar and that the present differentiation between these will disappear.

Under REACH about 30 000 existing chemicals used on the European market, so-called phase-in chemicals, must be registered within a period of 11 years. The registration deadlines within this 11 year period are based on tonnage and hazardous properties (Figure 1). For new chemicals the registration under REACH starts on 1 June 2008 after a transition period of one year during which the old notification regime is kept in place.

With the REACH regulation in force, we feel that it is necessary to focus on some developments in the field of intelligent testing strategies. Intelligent testing strategies are integrated approaches comprising of multiple elements aimed at speeding up the risk assessment process while reducing costs and animal tests (1). REACH, in its Annex XI, gives the opportunities to deviate from the standard test packages required for the different tonnage bands according to Annex VII to X (Figure 2). The present document will focus on read-across and the inter-relationship between read-across, grouping and (Q)SAR ((Quantitative) Structure Activity Relationship). These three approaches are so-called non-testing methods (RIP 3.3.1 TAPIR project).

In order to understand the inter-relationship between the three non-testing methods, it is necessary to define each of them accurately.

A **chemical category** is a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity (REACH Annex XI/OECD (2)). Group members can have a similar metabolite or precursor, a similar functional group in the molecule or a constant pattern in the changing of properties within the group.

(Q)SARs are theoretical models that relate chemical structure to a physicochemical property, environmental fate parameter, toxicological or ecotoxicological effect (OECD). REACH Annex XI adds the following prerequisites:

- results are derived from a (Q)SAR model whose scientific validity has been established,
- the substance falls within the applicability domain of the (Q)SAR model,
- results are adequate for the purpose of classification and labelling and/or risk assessment, and

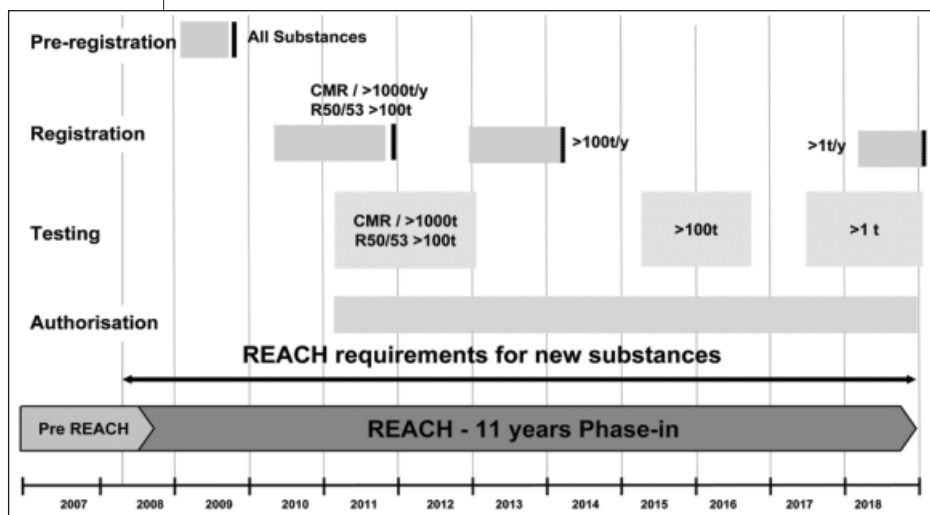


Figure. 1 REACH timeframe

Annex	Phys chem data	Toxicological data	Ecotoxicological data
VII	<ul style="list-style-type: none"> State of substance at 20°C Melting/freezing point Boiling point Relative density Vapour pressure Surface tension Water solubility Partition coefficient n-octanol / water Flash point/flammability Explosive properties Self-ignition temperature Oxidising properties Granulometry 	<ul style="list-style-type: none"> Skin irritation/corrosivity <i>in vitro</i> tests Eye irritation <i>in vitro</i> tests Skin sensitization evaluation or local lymph node assay Ames test Acute oral toxicity 	<ul style="list-style-type: none"> Acute <i>Daphnia</i> toxicity Algae growth inhibition Ready Biodegradation
VIII		<ul style="list-style-type: none"> <i>In vivo</i> skin irritation <i>In vivo</i> eye irritation <i>In vitro</i> gene mutation assay <i>In vitro</i> chromosome aberration test Acute inhalation/dermal toxicity 28-day repeat-dose study Reproductive/Developmental toxicity screening study Toxicokinetic assessment 	<ul style="list-style-type: none"> Acute fish toxicity Activated sludge respiration inhibition test Further biodegradation Hydrolysis test Adsorption/desorption screening test
IX*	<ul style="list-style-type: none"> Stability in organic solvents Dissociation constant Viscosity 	<ul style="list-style-type: none"> <i>In vivo</i> mutagenicity studies 28-day or 90-day repeat dose study in the rat Pre-natal development toxicity study Two-generation reproductive toxicity study 	<ul style="list-style-type: none"> Long-term <i>Daphnia</i> toxicity Long-term fish toxicity Simulation test on the ultimate degradation in surface water Soil simulation test Sediment simulation test Identification of degradation products Fish bioaccumulation study Further adsorption/desorption study Short term toxicity to invertebrates (earthworm) Study of the effects on soil micro-organisms Short-term toxicity to plants
X*		<ul style="list-style-type: none"> Further mutagenicity studies Long-term repeat-dose (> 12 months) study in the rat Further toxicity study to investigate specific concerns Two-generation reproductive toxicity study in the rat Developmental toxicity (OECD 414) Carcinogenicity study (often combined with the previous study, usually in the rat) 	<ul style="list-style-type: none"> Further biodegradation in water, sediment, soil-covering degradation rate and identification of relevant degradation products Further environmental fate and behaviour studies Long-term earthworm toxicity Long-term toxicity to other soil invertebrates Long-term plant toxicity Long-term toxicity to sediment organisms Long-term or reproductive bird toxicity

* Test plan required

Figure 2. Tests as required according to Annex VII to X of REACH

– adequate and reliable documentation of the applied method is provided.

Read-across means according to the OECD: the use of data available for some members of a category to estimate values (qualitatively or quantitatively) for category members for which no such data exist.

Especially from the definition of read-across it becomes clear that grouping (or category approach) and estimation ((Q)SAR) can not be regarded separately from read-across, but are in fact more or less part of it. At present the concept of non-testing is poorly developed within the EU. Chemical categories were extensively used in the US-HPV Challenge program and also in the OECD/ICCA initiative to minimize the number of tests required. Guidance on how to apply grouping is available from these HPV programs. In the EU there is some experience with new substances, but this is not laid down in an EU guidance document. Some national authorities have developed guidance, which is not formerly accepted by other member states (3). Nevertheless grouping is becoming more popular and accepted within the EU and this can be considered one of the benefits of the upcoming REACH regulation.

QSARs are used to a very limited extent within the EU. They are mainly used as screening tool (e.g. in strategies for skin- and eye irritation testing) and not as a stand-alone approach. This is caused by the fact that QSAR results are accepted in a worst case approach only, because no formally validated and generally accepted QSARs exist at present. QSARs are developed to predict properties of a substance within the "borders" of a group, i.e. the applicability domain. Substances falling outside the applicability domain of the QSAR can not be accurately predicted, because no data exist to substantiate the accuracy of the QSAR. Within OECD, JRC and ECVAM at present much effort is placed in QSAR development and validation (A). It is clear that a formal validation according to the principles of the OECD is not feasible for the use of QSARs under REACH. Therefore, it is likely that a more pragmatic approach for the use of QSARs will have to be chosen. Read-across can be considered as the link between group members based on structural similarities. Thus, for read-across, a group (of at least 2 substances) and structural similarities between group members are essential. In addition, according to the definition data, need to be available for some category members. Although not further elaborated within the definition, the data are likely

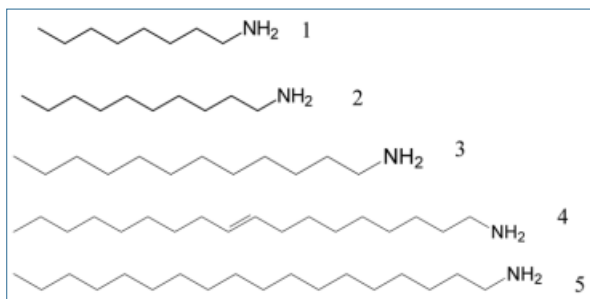


Figure 3. Grouping example

to be test data. These data can be used to estimate properties of group members without test data (read-across). In fact this mean that a structure activity relationship between group members is established, which is not quantified. Thus SAR not QSAR!

In the definition on read-across of the OECD no prerequisites are made for which substances within the group test data should be available and whether or not read-across is possible to all group members either by interpolation or by extrapolation. This differs from the approach which is likely to be chosen under REACH, where it is clearly stated that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (Annex XI).

As an example a group of primary amines is chosen to show the concept (see Figure 3). The substances have structural similarities with the amine group as functional group and a regular within group pattern (increasing carbon chain length). Assuming there are data on substances 1 and 3, a read-across to substance 2 would be possible. Substances 4 and 5 would fall outside the category (no extrapolation!) and to include them it would be necessary to generate data to allow interpolation. When data are available on substances 1 and 5, the read-across might be possible for the whole group (with some additional reasoning for the influence on effects of the saturation in molecule 4). In all cases the category approach has to be based on scientific arguments based on knowledge of chemistry, toxicokinetics and toxicology. For the non-tested substances within the group SAR could be applied, i.e. estimating a range of values for those endpoints with no test data available. Development of a QSAR is in most cases not necessary, because the ranges derived will fulfill requirements for classification and labeling and risk assessment. Sometimes, however, a range might be insufficient to meet these requirements; e.g. when one of the substances does not fall within the group pattern or the group pattern falls within different classification ranges (interruption of the group). In these cases the consequence is either to apply a worst case assumption (e.g. classify the whole group as dangerous) or to initiate additional testing to identify whether the SAR is accurate or not. In the case of a group of substances the applicability domain is clear and substantiated by data on substances at the borders of the category.

As stated above some experience with the use of categories and read-across is available within the context of new substances notifications. In the approach chosen, limited test data on all substances within a category are required, including

physicochemical data (to substantiate the category), acute toxicity data and a test for mutagenicity in bacteria (Ames test) (3). In some cases in addition a test with daphnia is required. This approach provides a good basis for a category and limits the risks of miss-prediction, while limiting the number of animals included in toxicity testing (B).

In a few months RIP 3.3 will provide guidance on how to approach non-testing methods under REACH. It is expected that the approach will be somewhere in between the OECD approach and the approach chosen for new substances. It seems reasonable to assume that in the non-testing approach scientific reasoning and expert judgement will play a significant role. In addition, the use of existing data, in vitro testing and weight of evidence approaches, might reduce the number of tests necessary to fulfil the requirements of REACH. Exposure based waiving of certain tests can even further reduce the number of tests and the costs associated with these tests.

It seems obvious that non-testing is attractive as an alternative to (vertebrate) testing, but a major issue might be that industry and authorities are not familiar with the approaches as described here and therefore are likely to prefer the standard test packages as mentioned in REACH for the different tonnages (Annex VII to X). Another issue might be that the development of an intelligent non-testing strategy might take more time than to the traditional method and this might influence its practical implementation. For industry it is important to know, that in a very early phase of REACH, the pre-registration, information on structural analogues (thus grouping/read-across) and (Q)SAR has to be provided as part of the information package. Although this is not a mandatory requirement, it seems clear that using information of data-rich analogues can be very attractive both from a financial and an ethical point of view. Therefore there is a clear task for science and authorities together with industrial organisations to promote and stimulate the use of alternative intelligent testing strategies as described here in this document.

REFERENCES AND NOTES

1. Van Leeuwen et al, 2005 Integrated testing strategies (www.ecb.jrc.it)
 2. OECD, 2005 Manual for Investigation of HPV Chemicals, 3.2 Guidance on the Development and Use of Chemical Categories in the HPV Chemicals Programme
 3. Hanway R. (2002). The use of toxicological read-across data in the notification of new chemicals. UK Health and Safety Executive, presented at the Technical Meeting, March 2002.
- A. Elements concerning validation of QSARs are listed in the OECD QSAR validation principles, amongst which are also requirement for definition of the applicability domain of the models.
- B. Only the acute toxicity study (using vertebrates) is necessary.

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