REACH - Minimisation of Animal Testing

This leaflet provides a brief summary of the opportunities that are available to registrants to minimise animal testing.

What is REACH?

REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) is the system for controlling chemicals in Europe. It became law in the UK on 1 June 2007. The European Chemicals Agency (ECHA) is the regulatory authority that manages the implementation of REACH.

Minimisation of animal testing

When considering how to fulfil the REACH information requirements, opportunities are available to registrants to minimise animal testing and reduce costs.

Over 14,000 substances have been registered to date (June 2016) and more are expected when the final phase-in deadline arrives in June 2018. For a single, high-tonnage substance, with no pre-existing data, and no attempt to minimise animal testing, registration and subsequent fulfilment of the information gaps could require over 5,000 animals (assuming little or no avian testing). The REACH Regulation provides opportunities to reduce costs and register effectively using fewer animals than predicted.

Article 13(4) of REACH stipulates that toxicological and ecotoxicological tests shall be carried out in compliance with EU Directive 86/609/EEC on animal protection. This Directive has been superseded by Directive 2010/63/EU, which provides basic requirements for the care and accommodation of laboratory animals, and stipulates that experiments shall be designed to reduce the duration and intensity of suffering to the animal. Furthermore, experiments on live animals shall not be performed if the results can be obtained by another scientifically satisfactory method. The Animals (Scientific Procedures) Act 1986 (as amended in 2012) transposes Directive 2010/63/EU into UK law, and provides the regulatory framework for controlling animal testing in the UK.

Where possible, scientifically sound approaches to the implementation of the 3Rs (reduction, refinement or replacement of animal use), which are already stipulated under the REACH Regulation, should be used. This is particularly important for the registration of the higher-tonnage substances, as it is very unlikely that complete replacements for some toxicity endpoints will be available. The Annexes in the REACH legislation (Annexes VII to XI) provide information and guidance on the adaption (“waiving”) of animal tests. ECHA has provided some further guidance to help; the most relevant is the REACH guidance on information requirements and Chemical Safety Assessment (CSA)[1].

Key messages for registrants

1 Before concluding that there is an information gap for toxicity, the Substance Information Exchange Forum (SIEF) should consider all the available existing information.

2 Consider whether animal testing can be minimised by following one or more of the specific testing adaptations provided in the REACH Regulation itself, see Part 1 (below).

3 If no specific adaptations are applicable, consider whether animal testing can be minimised in other more general ways *in-lieu* of testing - see Part 2 (below).

4 If adaptation is not possible, and a new animal test is required, ensure that the test of least severity and using the fewest animals is employed. The test should be that expected to cause the least pain, suffering, distress and lasting harm.

These are illustrated in the diagram overleaf:

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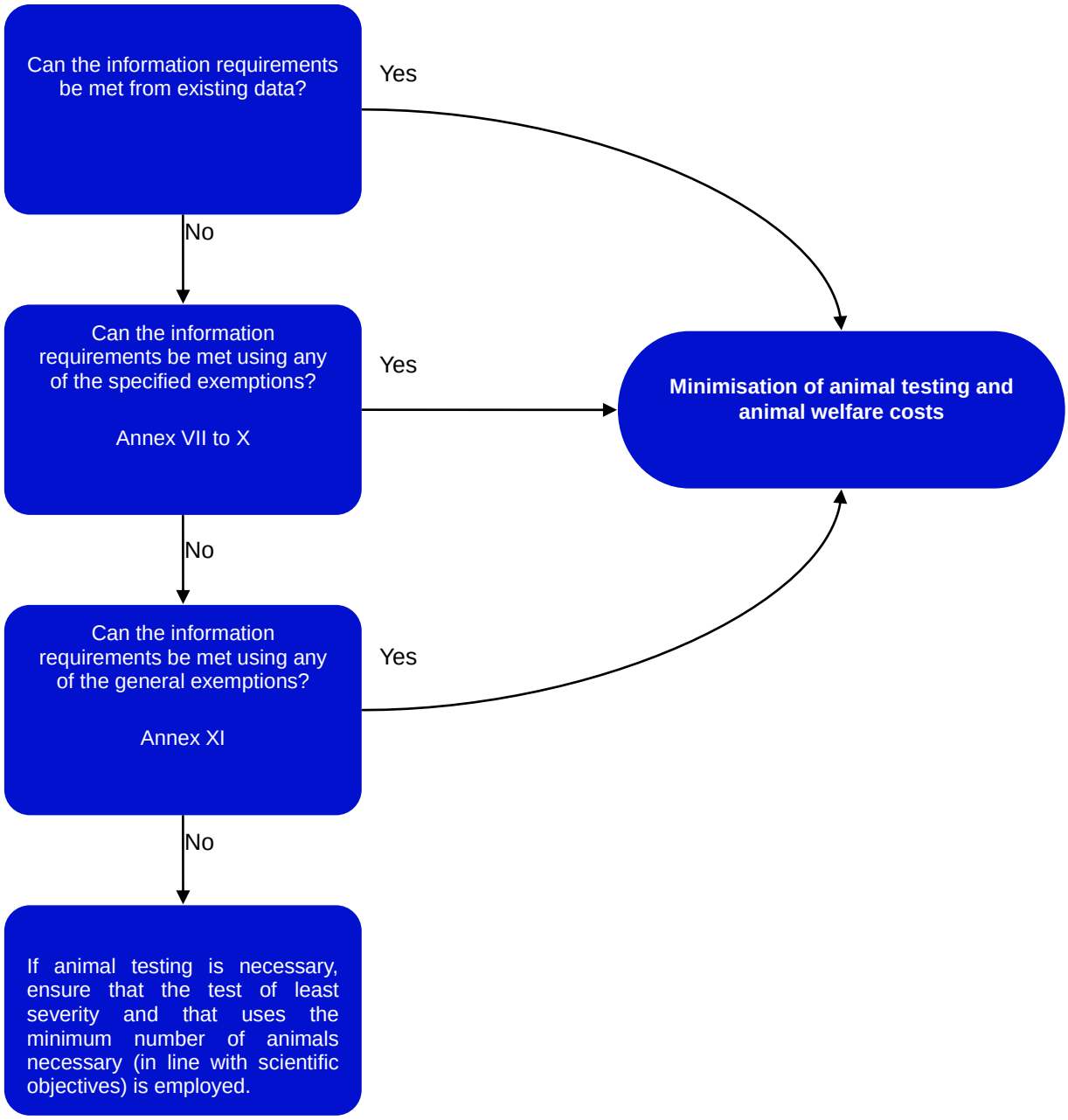

The UK Competent Authority recommends that the duty holders who are compiling registration dossiers consider whether these approaches to the minimisation of animal testing will be of relevance to them.

Part 1: Opportunities for minimising new animal testing
REACH Annexes VII-X

The standard REACH information requirements can be found in column 1 of the tables in Annexes VII to X. In column 2 there are rules for adaptation of the tests specified in column 1. These rules outline circumstances in which a particular information requirement involving a vertebrate-animal test may be modified. There are many specific rules, and some of the key ones are detailed in Table 1, below. Most significantly, there are derogations for: postponement of reproductive toxicity (fertility) testing, and circumstances for waiving of the short-term repeated dose and reproductive toxicity screening studies.

The validation status of alternative tests is subject to regular change, and those commissioning tests should take account of this by checking the current status.

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Table 1 Key opportunities for minimising new animal testing (Annex VII to X)

Opportunities to adapt tests - Annex VII (1-10 tonnes/annum)	
Skin Sensitisation	Non-animal testing will soon be the standard requirement. An amendment to Annex VII of REACH to reflect this change is expected in Autumn 2016. Draft guidance on the information requirements for this endpoint can be found on the ECHA website[2]. Where an *in vivo* test is required, the study of choice is the Local Lymph Node Assay (LLNA – OECD TG 429), with justification needed for guinea pig tests. It is possible to use the reduced LLNA protocol as described in TG 429, but this does not give information on potency. Similarly, the two non-radiolabelled variants of the LLNA (OECD TGs 442A/B) do not currently provide information that could inform on potency.
Acute Oral Toxicity	The test does not need to be conducted if the substance is corrosive, or if an acute inhalation toxicity study is available. Weight of evidence (WoE) assessment (as described in Annex XI): • The *in vitro* NRU3T3 test investigates toxicity via the oral route. This information can be used as part of a WoE assessment to waive the requirement for an acute oral toxicity study or inform on the appropriate dose. • It may be possible to use information from existing repeated dose studies to inform on the acute toxicity of the substance. Recent analysis showed that, in most cases, substances found to be of low toxicity in oral sub-acute studies of at least 28 days duration (NOAEL ≥ 1000mg/kg bw) are predicted to be not classified for acute oral toxicity (i.e. LD_{50} > 2000mg/kg bw). Therefore, if a repeated dose study gave a NOAEL ≥ 1000mg/kg bw, it may be possible to include this, as part of a WoE assessment, to waive the requirement for an acute oral toxicity test.
Skin sensitisation and Acute Oral Toxicity	Annex III provides the opportunity for some substances to be registered at 1-10 tonnes per annum with a reduced set of information requirements. For further details see our leaflet on Annex III.
Opportunities to adapt tests - Annex VIII (10-100 tonnes/annum)	
Acute Dermal Toxicity	In addition to the Annex VII requirement for Acute Oral Toxicity testing, Annex VIII requires that the acute toxicity of the substance is tested via a second route of exposure (dermal or inhalation). The route chosen is dependent on the nature of the substance and the likely route of human exposure. For substances that are non-toxic via the oral route, the dermal test may be waived since these substances can be expected, with a high degree of certainty, to be non-toxic following dermal exposure.
Skin irritation	The Organisation for Economic Cooperation and Development (OECD) has agreed on test guidelines for alternative methods, which have been included in the Test Methods Regulation (440/2008/EEC). Annex VIII has been updated to reflect these advances; with the standard information requirement now being for the *in vitro* studies. The *in vitro* test guidelines which have been adopted are: • OECD 439 – *in vitro* skin irritation (revised in 2013) • OECD 431 – *in vitro* skin corrosion (revised in 2013) • OECD 430 – Transcutaneous electrical resistance test (TER) (revised in 2013) • OECD 435 – *in vitro* membrane barrier test method (2006) *In vivo* testing is only required in exceptional circumstances (please refer to REACH Annex VIII 8.1 column 2).

Eye irritation	Annex VIII has also been updated in relation to the requirements for eye irritation. The standard information requirement is now for the *in vitro* tests. The *in vitro* test guidelines which have been adopted are: • OECD TG 437 – The Bovine Corneal Opacity and Permeability test method (BCOP) (revised in 2013) • OECD TG 438 – Isolated Chicken Eye Test (ICE) (revised in 2013) • OECD TG 460 – Fluorescein leakage (FL) method (2012) • OECD TG 491 – Short Time Exposure (STE) (2015) • OECD TG 492 – Reconstructed human Cornea-like Epithelium (RhCE) (2015) The draft guideline for the Cytosensor Microphysiometer (CM) Test Method is under discussion at the time of writing. *In vivo* testing is only required in some circumstances (please refer to REACH Annex VIII 8.2 column 2).
Repeat dose and reproductive toxicity	If both repeated dose toxicity and reproductive toxicity studies need to be conducted: (a) For substances up to 100 tonnes/annum: conduct the "combined" study (OECD TG 422) to save animal numbers. (b) For substances at 100 tonnes/annum or more: propose standard reproductive and developmental toxicity studies, and long term repeated dose toxicity studies, and do not conduct any shorter-term studies for these end points. However, the CSR should indicate the interim risk-management measures downstream users should take before the findings from the proposed studies are included.
Short-term toxicity to fish	Testing does not need to be conducted if the substance has very low solubility in water or is unlikely to cross biological barriers. Or if a long-term fish toxicity study is available. The threshold approach for acute fish toxicity should be used where possible, if a test is needed[3]. You should also consider whether a fish embryo toxicity test (OECD TG 236) would fulfil the information requirement, taking account of the recommendations in ECHA (2016)[4].
Opportunities to adapt tests - Annex IX (100-1000 tonnes/annum)	
Reproductive study	The requirement for a fertility study (OECD TG 443) should be considered at 100 tonnes/annum (Annex IX) if adverse effects on the reproductive tissues are observed in the available repeated dose studies, or screening studies for reproductive effects. Where no such adverse effects are observed, the fertility study would only need to be conducted if the tonnage reached 1000+ tonnes/annum.
Opportunities to adapt tests - Annexes IX and X (100 and 1000 tonnes/annum)	
Extended One-Generation Reproductive Toxicity Study (EOGRTS) OECD TG 443	The standard information requirement in Annexes IX and X is limited to the basic design of the EOGRTS (i.e. F1 generation only). The additional cohorts for developmental neuro- and developmental immunotoxicity- testing and production of the f2 pups are triggered depending on a weight of evidence assessment (please refer to REACH Annexes IX and X 8.7.3 Column 2).
Long-term fish toxicity	Need not be proposed unless required to clarify risks.
Bioaccumulation in fish	Need not be proposed if the substance has a low potential for bioaccumulation (for instance a log Kow < 3) and/or a low potential to cross biological membranes; or

	– direct and indirect exposure of the aquatic compartment is unlikely The aquatic exposure test permits modifications that can reduce the use of animals in some circumstances (a minimised design, or a test with a single concentration). Further guidance about when either modification may be appropriate is under development by the OECD.
Long-term reproductive toxicity to birds	Need not be proposed if risks to predatory birds can be assessed using mammalian toxicity data

Note: more detailed information is available in the REACH endpoint specific guidance[5].

Part 2: Opportunities for minimising new animal testing – REACH Annex XI

The UK REACH Competent Authority encourages duty holders to consider the extent to which Annex XI of REACH might apply to them. This Annex provides general rules that registrants may use to adapt ("waive") the standard testing regime set out in Annexes VII to X and thereby minimise new animal testing. These rules can be applied individually or in a weight of evidence argument *in lieu* of new animal testing.

For Annexes VII and VIII, the adapted information should be included directly in the registration dossier, and will not be compliance-checked routinely by ECHA. However, for Annexes IX and X it is a Testing Proposal (including the proposal for adaptation) that will be required, rather than the adapted information itself. These Testing Proposals will be subject to a formal evaluation by ECHA, who will judge whether an adaptation or a standard test should eventually be submitted.

Use of existing data

REACH requires that new studies should be conducted in a Good Laboratory Practice (GLP) accredited laboratory. However, the same standard does not apply to pre-existing studies, i.e., pre existing studies need not have been conducted in a GLP environment. The minimum requirement is that the study is of sufficient quality to enable a decision on classification and labelling to be made and/or a CSA to be conducted. A useful "rule of thumb" in judging quality is to look at the relevant international standard (OECD[6] or EU[7]) and consider how closely the old study matches. It should be noted that if an old study does not reveal a hazard it should not automatically be disregarded.

It has been suggested that it may be possible to estimate a $LD(C)_{50}$ value (a dose or concentration that kills half the test animals after a single exposure) from existing repeated dose studies (28/90-day studies) if the relevant acute toxicity study is missing. In principle this is possible, but only if mortalities are observed during the early part of the study, say after 2-3 exposures/doses. The later in the study mortalities are observed, the more difficult it becomes to consider them to be acute lethality. The same approach could be taken with early clinical signs of toxicity, which could be used to estimate the level of evident toxicity, which is the stopping point for the fixed dose procedure (OECD TG 420). The critical factor is that the clinical signs should be observed very early in the study.

Although *in vitro* tests are now the standard information requirement for skin irritation/ corrosion and eye irritation/ damage in Annex VIII, it is still possible to fulfil the data requirement for these hazard classes by using information from **existing** *in vivo* studies.

Weight of evidence assessment

This is a common scientific practice which can be applied when a conclusion on the presence/absence of a hazardous property cannot be made on information from a single source alone. For example, there could be information from a number of studies, none of which were conducted to recognised international standards. However, when taken together, these can be used to meet the REACH information requirements and be sufficient for a CSA.

A weight of evidence approach is a very efficient way of maximising the potential of older studies, and avoiding new animal testing for a particular hazardous property.

(Quantitative) structure activity relationships - (Q)SARs

(Q)SARs are computer-based models which are designed to predict the physico-chemical properties, the potential human health effects or the environmental effects of a substance. This is done from knowledge of its chemical structure. In principle, the results from internationally accepted (Q)SARs could be used to fulfil one or more of the REACH information requirements or help build chemical categories without the need to use animal tests. Further information on the application of (Q)SARs can be found in Chapter 6 of the REACH guidance on information requirements and chemical safety assessments8.

A useful repository of acceptable (Q)SARs for use by potential registrants is the OECD (Q)SAR application toolbox9 which has been jointly developed by the OECD and ECHA. The toolbox is regarded as an essential tool for helping registrants obtain relevant information on the intrinsic hazards of their substances.

On a cautionary note however: the substance in question should fall in the "applicability domain" of the model; the results will have to be adequate for the purpose of classification and labelling and/or risk assessment; and adequate and reliable documentation of the model will need to be provided. It is anticipated that (Q)SARs will play an increasing role for registrations in 2018, as information from earlier registrations is used to refine the existing (Q)SARs.

Grouping and read across

Where substances share structural similarities or share common metabolic pathways, they can be grouped together in a chemical category. Categories of chemicals are selected on the assumption that the properties of a series of chemicals with common structural features will show similar trends in their physico-chemical properties, and more importantly, in their toxicological (human health/ecotoxicity) effects or environmental fate properties. Common behaviour or consistent trends are generally associated with a common underlying mode of action.

Once a group has been established, it is possible to use information from the data rich members to fill data gaps by the process of read-across. This can be either qualitative, in which the presence (or absence) of a particular hazard is predicted, or quantitative in which the magnitude of a particular property is predicted; for example, a No Observed Adverse Effect Level (NOAEL– a dose or concentration at which no adverse changes are observed).). It is important to ensure the category has a scientifically sound basis and that all hazards are read across, both positive and negative.

There is a lot of experience of developing and applying chemical categories in a regulatory context, for example in the OECD High Production Volume Chemical (HPV) programme. This experience has been further developed by ECHA, with guidance and examples now available on their website[8].

ECHA has developed a Read Across Assessment Framework (RAAF) which outlines how they systematically assess read across and grouping approaches used to address human health hazards in registration dossiers. It is recommended that registrants consider the RAAF when using a read across and grouping approach. More information can be found on ECHA's website[10].

At the time of writing, a RAAF for environmental endpoints is in development.

In vitro methods

In some instances, information from *in vitro* tests may be used for hazard identification and as part of a CSA (REACH Annex XI 1.4). For a positive outcome only, a test is considered acceptable if it meets the European Centre for the Validation of Alternative Methods (ECVAM[11]) criteria for acceptance into the pre-validation process. For negative outcomes to be accepted; the test must have been scientifically validated, the results should be adequate for classification and labelling and/or use in a CSA, and adequate documentation is available.

Where *in vitro* tests have been successfully validated and adopted into the REACH Test Methods Regulation (EC 440/2008), these should be used in preference to the equivalent *in vivo* test.

For skin irritation/corrosion and eye irritation/damage, Annex VIII has been revised so that *in vitro* tests are now the standard information requirements for these endpoints. A revision of Annex VII (expected to

be published in Autumn 2016) will specify that non-animal tests are the default requirement for Skin Sensitisation.

Testing is not technically possible

It is possible to adapt some tests if it is not technically possible to conduct the test, or the substance is very hazardous; for example, due to the generation of an explosive atmosphere. The REACH Test Methods Regulation should also be consulted, as some test methods, such as the acute dermal and acute inhalation tests identify specific circumstances where testing is inappropriate.

Exposure-based waiving

Exposure-based waiving of certain tests may be permitted in cases where it can be shown that exposure is insignificant or absent for the substance concerned. This will depend on the conditions of use and is outside of the scope of this document.

Application of the 3Rs (reduction, refinement and replacement)

Directive 2010/63/EU indicates that procedures should not be carried out "if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union. In choosing between procedures, those which to the greatest extent meet the following requirements shall be selected:

(a) use the minimum number of animals;
(b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm;
(c) cause the least pain, suffering, distress or lasting harm; and are the most likely to provide satisfactory results.

This means that where there is a choice of tests for a particular end point, the test of least severity should be used. This applies to acute oral toxicity where the Fixed Dose Procedure (OECD TG 420) offers some animal welfare advantages over the other available test methods. The development of in-vitro testing for skin irritation/corrosivity and eye irritation/damage has progressed to the point where validated OECD Test Guidelines are available and have been included in the revised Annexes of REACH, making in vitro tests the standard information requirement for these endpoints. Similar advances in alternatives for skin sensitisation studies mean that non-animal methods will be the default information requirement for this endpoint (A revision of Annex VII is expected to be published in Autumn 2016).

Further information

Detailed guidance is available on ECHA's REACH website and further information from the UK Competent Authority can be found at (http://www.hse.gov.uk/reach/).

For general advice on the application of REACH obligations, you can contact the UK REACH Competent Authority's helpdesk by email: UKREACHCA@hse.gov.uk

Website links referred to in this leaflet

1. **REACH Guidance on Information requiremens and Chemical Safety Assessments (CSA)** http://echa.europa.eu/documents/10162/13643/information_requirements_r2_en.pdf

2. **Draft guidance on the information requirements for skin sensitisation:**

   http://echa.europa.eu/view-article/-/journal_content/title/registrants-to-use-alternative-test-methods-for-skin-sensitisation

3. **OECD – The Treshold Approach for Acute Fish Toxicity Testing**

   http://www.oecd.org/chemicalsafety/testingofchemicals/40985084.pdf

4. **Analysis of the relevance and adequateness of using Fish Embryo Acute Toxicity (FET) Test Guidance (OECD 236) to fulfil the information requirements and addressing concerns under REACH**

http://echa.europa.eu/documents/10162/13639/fet_report_en.pdf

5. **REACH Endpoint Specific Guidance*:**
   http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

   ***This guidance is being updated at the time of writing. To keep up to date, please check the ECHA wesbite**: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

6. **OECD:**
   http://titania.sourceoecd.org/vl=1350577/cl=25/nw=1/rpsv/periodical/p15_about.htm?jnlissn=1607310x

7. **European Union – REACH Test Methods Regulation: (EC) No 440/2008**

   http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0739:EN:PDF

8. **REACH Information requirements:**

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

9. **OECD (Q)SAR Toolbox:**

   http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html

10. **Information on grouping of substances and read across:**

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

11. **ECVAM:**

    http://ecvam.jrc.it/