ChemicalWatch

Avoidable animal tests continue under REACH

Neither companies nor ECHA are considering animal testing as a last resort. The agency must up its game, and the REACH annexes and relevant guidance must be updated fast

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As the largest animal testing programme in the world, the REACH Regulation has already consumed approximately 800,000 animals, with millions more expected to be used in the coming years. The key principles underlying REACH are to protect human health and the environment and to promote non-animal testing methods for the purposes of substance hazard assessment. It is with this last principle in mind that ECHA, the agency that oversees REACH, publishes a triennial report on the use of alternative test methods for the programme (sometimes referred to as the Article 117 Report), the second of which was published in June (CW 3 June 2014).

To minimise new animal testing, REACH contains a number of specific measures and general provisions designed to establish and enforce the principle that animal testing should only be performed as a last resort. For example, registrants are required to submit testing proposals to ECHA – usually for tests using the largest numbers of animals – for approval, before the test is conducted. Furthermore, non-testing methods such as read-across and weight-of-evidence approaches and non-animal testing methods must be used wherever possible.

The first Article 117 Report, published in 2011, highlighted a number of areas in which animal tests were conducted without prior approval or where an alternative method was available. It was clear that ECHA was not fulfilling its mandated role of ensuring that registrations are compliant with the last-resort principle. In 2012, the European Ombudsman launched an enquiry into the actions of ECHA, following a complaint submitted by the People for the Ethical Treatment of Animals Foundation (Peta) UK, alleging that ECHA was failing to ensure that alternative methods were being used wherever possible. The recent publication of the 2014 report reveals a continuation of these same failings.

No prior approval

The 2014 Article 117 report revealed that 167 tests had been conducted without prior approval of a testing proposal and with no appropriate justification. This resulted in an estimated 100,000 animals being used, which could have been avoided. Furthermore, these tests were conducted without the opportunity for third parties to submit additional data or propose testing strategies. To date, most third party comments were from animal welfare groups with accredited ECHA stakeholder status; therefore, the impact of such comments is potentially of great significance. It is essential that ECHA informs EU member states to follow up on all potential breaches of the last-resort principle and asks registrants to submit further information to justify the tests. Without penalties for conducting tests that lack prior approval, this breach of the REACH Regulation will continue.

Delays in accepting and implementing alternative methods

The lag between acceptance of new validated methods and integration into the EU test method Regulation and REACH annexes is likely to be responsible, in part, for the failure of registrants to use non-animal methods. Since 2009, 291 new skin corrosion/irritation tests have been conducted on approximately 873 rabbits, and the majority (57%) of all new eye irritation tests used rabbits, despite the availability of validated *in vitro* methods adopted by the OECD. Members of the Peta International Science Consortium, Ltd. (PISC) have been told by some companies that they believe *in vivo* testing is still required for skin and eye irritation and corrosion. This misunderstanding is almost certainly a direct consequence of the listing in Annex VIII (which sets out the information needed for substances made/imported in volumes above ten tonnes) of *in vivo* testing for both skin and eye irritation and corrosion in Column 1, despite the acceptance of non-animal methods for both positive and negative classifications.

Furthermore, ECHA's endpoint-specific guidance documents for these endpoints are shamefully out of date. It is essential that ECHA fulfils its responsibility to verify that validated *in vitro* methods are fully implemented. The European Commission must update the REACH annexes and require the agency to update its endpoint-specific guidance, before thousands of animals are used in avoidable testing. An up-to-date list of approved non-animal testing methods can be accessed here.

The limited uptake of *in vitro* tests for skin sensitisation is extremely disappointing, with only 7% of new tests being conducted this way. In 2012, the OECD published the *Adverse Outcome Pathway for Skin Sensitisation,* which describes the key events in an adverse response from the molecular initiating event to adverse health effects in humans. Several *in vitro* methods, validated by the European Union Reference Laboratory for Alternatives to Animal Tests (EURL Ecvam), are available to predict numerous steps in the pathway. Used in conjunction with Qsars (quantitative structure activity relationships) and other non-testing methods, there is simply no justification for registrants to continue using animals for skin sensitisation testing. ECHA says guidance on how to apply *in vitro* methods to predict the skin sensitisation potential of a chemical will be available by 2018 – by which time as many as 200,000 animals may have already been killed in skin sensitisation tests. This timeline is entirely unacceptable, and the Commission must dedicate adequate resources to ensure that guidance is available well in advance of the 2018 deadline.

The 2014 Article 117 report says acute toxicity is one of the endpoints for which existing and new *in vivo* experimental studies are used most often to address the information requirements. This trend is of particular concern as registrants prepare for the 2018 deadline – less data existed for registration dossiers covered by Annex IX (100 tonnes or above) compared with those covered by Annex X (1,000 tonnes or above).

If this trend continues for Annex VII (one tonne) and VIII dossiers, an unprecedented number of acute toxicity tests could be conducted in the coming years. It is essential that the REACH annexes are urgently updated to allow use of the EURL Ecvam–recommended 3T3 neutral red uptake (NRU) assay method to classify non-toxic chemicals; correspondingly, use of the *in vivo* assay should only be allowed when perceived to be absolutely necessary (for example, in the case of a positive result in the 3T3 NRU assay). The most relevant route of exposure should be used, with no Annex VIII requirement for a second administration route.

Both registrants and ECHA must embrace *in vitro* methods and take responsibility for ensuring that animal testing is minimised. PISC recently urged the European Commission to ensure that the EU test method Regulation and REACH annexes are urgently updated with the most current OECD test guidelines and methods recommended by EURL Ecvam, and that requirements for obsolete animal tests are removed. Our organisation also urged the Commission to ensure that ECHA update and consolidate its guidance and provide more useful materials for registrants. Doing so will avoid confusion over the use of adaptations outlined in the REACH annexes and help avoid animal testing wherever possible.

Read-across

Read-across is the mechanism by which the largest number of animal tests can be avoided in compliance with REACH, and it is encouraging that 75% of dossiers contain read-across arguments or a category approach for at least one endpoint. However, ECHA's narrow interpretation of read-across is probably preventing some registrants from using this approach; even cases that are clearly scientifically justified have been rejected.

PISC conducted a systematic review of dossier evaluation decisions on the use of read-across and revealed that ECHA is overly conservative in its acceptance of the method (GBB September 2013). ECHA only accepts interpolation (GBB October 2013) of data from reference chemicals and rejects data

How to minimise animal testing for Annex VII and VIII dossiers

Skin irritation and corrosion

» Use OECD-accepted *in vitro* methods in an integrated testing strategy to predict both skin irritation and corrosion for most chemical classes and, importantly, predict non-irritancy. *In vivo* testing is not required under Annex VII or VIII.

OECD test guidelines (TGs) include:

» OECD TG 430: *in vitro* skin corrosion: transcutaneous electrical resistance test method

» OECD TG 431: *in vitro* skin corrosion: reconstructed human epidermis test method

» OECD TG 435: *in vitro* membrane barrier test method for skin corrosion

» OECD TG 439: *in vitro* skin irritation: reconstructed human epidermis test method

Eye irritation and corrosion

» Use OECD-accepted *in vitro* methods in an integrated testing strategy to completely eliminate animal testing for eye irritation and corrosion and, importantly, predict non-irritancy. *In vivo* testing is not required under Annex VII or VIII.

OECD TGs include:

» OECD TG 460: fluorescein leakage test method for identifying ocular corrosives and severe irritants

» OECD TG 437: bovine corneal opacity and permeability test method

» OECD TG 438: isolated chicken eye test method

» In addition to the OECD TG, the cytosensor microphysiometer method is recommended by the European Union Reference Laboratory for alternatives to animal testing to differentiate water-soluble ocular corrosives and severe irritants and non-irritants in top-down and bottom-up approaches, respectively.

» Draft OECD TG: the short time exposure *in vitro* test method for i) identifying chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage*

Skin sensitisation

» Use the direct peptide binding assay* (draft), KeratinoSens™* (draft), human cell line activation test and Qsars (for example, the OECD Qsar toolbox**) in an integrated testing strategy or weight-of-evidence approach to predict skin sensitisation.

Mutagenicity

If *in vitro* testing under Annex VIII triggers *in vivo* mutagenicity testing, always submit a testing proposal for prior approval by ECHA.

In vitro OECD TGs include:

» OECD TG 471: bacterial reverse mutation (Ames) test (1997)

» OECD TG 476: *in vitro* cell gene mutation test in mammalian cells (1997)

» OECD TG 473: *in vitro* chromosomal aberration test in mammalian cells (1997)*

» OECD TG 487: *in vitro* mammalian cell micronucleus test (2010)*

» OECD Qsar toolbox

Acute toxicity

» Use the 3T3 neutral red uptake cytotoxicity test to predict non-toxic chemicals.

» Use exposure-based waiving arguments or ADME information to avoid testing via multiple routes of administration.

» OECD Qsar toolbox

Short-term aquatic toxicity

» Use the fish embryo toxicity test (OECD TG 236) to predict fish acute toxicity.

» OECD Qsar toolbox

Notes:

* Some OECD documents linked here are draft or have been updated but the final versions are not yet available. Please check the OECD website for the most updated versions of all documents.

**Other Qsar tools available here.

extrapolation strictly according to the REACH legal text under Annex XI. The agency guidance for read-across and categories expressly stipulate the use of interpolation but not extrapolation. Furthermore, the few cases that have been taken to ECHA's Board of Appeal in relation to read-across have been rejected on the grounds that the extrapolated data did not meet the conditions for a read-across adaptation to be accepted. Yet, extrapolation is critical for reducing animal use and has been accepted as a scientifically valid method; as highlighted in the ECHA Qsar and grouping guidance during an experts workshop on read-across assessment that was organised by the agency, as well as by the OECD in its guidance on the grouping of chemicals.

The agency has often called for registrants to provide a thorough scientific justification and rationale, when proposing the use of read-across as a method to fulfil data requirements. However, this issue is a complex one for industry to contend with, as the agency's restrictive approach to read-across appears to be a work-in-progress, with only one illustrative example published. Furthermore, the agency's highly anticipated read-across assessment framework, expected in 2014, has yet to be published. With registrants already starting to fill data gaps in preparation for the 2018 deadline, it is essential that ECHA provides adequate guidance as a matter of urgency and that registrants present a robust justification when proposing read-across arguments.

Third party consultations

Further exacerbating the situation is the fact that, even though ECHA concedes that information provided by third parties may be scientifically valid, it refuses to consider testing strategies proposed by third parties – leading again to animal testing that might have been avoided. The agency must take responsibility for ensuring that animal testing is conducted only as a last resort, by requesting alternative testing strategies once they have come to light; to do otherwise would be unethical and in direct violation of the underlying principle of REACH – to promote non-animal testing methods.

Animal use must be minimised

It is evident that, under REACH, the principle of using animals only as a last resort for testing is not being adequately adhered to by registrants or ECHA. Tests continue to be conducted without prior approval, and animals continue to be used where valid alternatives exist.

To ensure that animal tests are conducted only as a last resort, it is essential that the agency fulfils its legal requirement when conducting compliance checks to verify if animal tests are being conducted in line with the spirit and content of REACH, and to investigate the reasons for low uptake of alternative methods, so as to ensure that they are used to the fullest extent under the law. It is imperative that REACH annexes and corresponding guidance documents, including endpoint-specific guidance, be updated and highlighted to industry as a matter of urgency to reflect the recent developments in non-animal methods and testing strategies. Likewise, registrants share the responsibility of adhering to the legal principle of using animals only as a last resort and seeking alternative methods wherever possible.

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