A Campaign to End Animal Testing: Introducing the PETA International Science Consortium Ltd

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Summary — The successful development and validation of non-animal techniques, or the analysis of existing data to satisfy regulatory requirements, provide no guarantee that this information will be used in place of animal experiments. In order to advocate for the replacement of animal-based testing requirements, the PETA International Science Consortium Ltd (PISC) liaises with industry, regulatory and research agencies to establish and promote clear paths to validation and regulatory use of non-animal techniques. PISC and its members use an approach that identifies, promotes and verifies the implementation of good scientific practices in place of testing on animals. Examples of how PISC and its members have applied this approach to minimise the use of animals for the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation in the EU and testing of cosmetics on animals in India, are described.

Key words: *animal testing, chemical testing, cosmetics testing, data requirements, OECD, PISC, REACH, Three Rs.*

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Introduction

The PETA International Science Consortium Ltd (PISC) — whose members are the People for the Ethical Treatment of Animals (PETA) UK, PETA US (the largest animal rights organisation in the world), PETA France, PETA Germany, PETA India, PETA Netherlands, PETA Asia and PETA Australia — was established in 2012 to coordinate the scientific and regulatory expertise of its members and to develop and promote strategies that will ultimately eliminate the use of animals in experiments. Using its diverse expertise and a multi-faceted approach for working with regulators, policy-makers and companies, PISC is able to address a broad range of issues, including the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) programme in the European Union (EU), and the testing of cosmetics and household products internationally. A primary focus of PISC is the international harmonisation of testing methods, as advances in one region are better served by assurances that those gains are meaningful elsewhere.

Minimising Animal Testing for REACH

The key principles underlying REACH are the protection of human and environmental health through substance hazard assessment and the promotion of non-animal testing methods to meet programme requirements. When the EU passed the REACH legislation in 2006, this programme was intended to ensure that animals would be used only as a last resort. Yet, so far, more than 800,000 animals have died in REACH tests (1), with millions more expected to be used in the coming years. Both REACH registrants and the European Chemicals Agency (ECHA; the agency responsible for implementing REACH) must embrace alternative methods and take responsibility for ensuring that animal testing is minimised and, therefore, PISC has given high priority to advocating these principles.

Providing registrants with advice and resources

Alternative methods are available that have been approved for use in place of animal experiments to meet REACH data requirements. With tens of thousands of substances due to be registered for the 2018 REACH deadline — many by small entities that have limited or no experience with REACH or the available array of alternatives to animal testing — it is essential that guidance on reducing animal testing be made available. With this in mind, PISC and *Chemical Watch* recently launched a free webinar series focusing on satisfying REACH requirements using non-animal testing strategies (2).

[a]*The author was a member of the 2013 Lush Prize judging panel.*

Table 1: How to minimise animal testing for REACH Annex VII and Annex VIII endpoints

Skin irritation/corrosion
If testing is required, registrants should use OECD-accepted *in vitro* methods, combined with an integrated approach to testing and assessment (IATA; 9), to predict both skin irritation and corrosion for most chemical classes and, importantly, predict non-irritancy. The legislative text for Annex VIII currently states that *in vivo* testing is required; however, *in vivo* testing can be waived if the test substance is within the applicability domain of the *in vitro* methods, as recognised in the ECHA guidance document (10). *OECD test guidelines include:* — OECD TG 430: *In vitro* transcutaneous electrical resistance test method for skin corrosion (11) — OECD TG 431: *In vitro* reconstructed human epidermis test method for skin corrosion (12) — OECD TG 435: *In vitro* membrane barrier test method for skin corrosion (13) — OECD TG 439: *In vitro* reconstructed human epidermis test method for skin irritation (14)
Serious eye damage and irritation
If testing is required, registrants should use OECD-accepted *in vitro* methods in an integrated testing strategy to eliminate animal testing for serious eye damage and, importantly, to predict non-irritancy. The legislative text for Annex VIII currently states that *in vivo* testing is required, however, this can be waived if the test substance is within the applicability domain of the *in vitro* methods and classified as causing serious eye damage or as a non-irritant as recognised in the ECHA guidance document (15). *OECD test guidelines include:* — OECD TG 460: Fluorescein leakage test method for identifying ocular corrosives and severe irritants (16) — OECD TG 437: Bovine corneal opacity and permeability test method (17) — OECD TG 438: Isolated chicken eye test method (18) — OECD draft TG: The short time exposure (19) *in vitro* test method for identifying i) chemicals inducing serious eye damage and ii) chemicals not requiring classification for eye irritation or serious eye damage — OECD draft TG: Reconstructed human cornea-like epithelium (RhCE) test method (20) for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage In addition to the OECD TG, the cytosensor microphysiometer method is recommended (21) by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) to differentiate water-soluble ocular corrosives and severe irritants and non-irritants in top-down and bottom-up approaches, respectively.
Skin sensitisation
Registrants should use the OECD Adverse Outcome Pathway for Skin Sensitisation (22), which describes the key events in an adverse response from the molecular initiating event to adverse health effects in humans. Several *in vitro* methods that have been validated by EURL ECVAM exist for predicting skin sensitisation and should be used in an integrated testing strategy or weight-of-evidence approach. In vitro *OECD test guidelines include:* — OECD draft TG: Direct peptide binding assay (23) — OECD draft TG: KeratinoSens™ (24) — OECD draft TG: Human cell line activation test (25) — OECD QSAR toolbox (26)
Mutagenicity
If *in vitro* testing under Annex VIII triggers *in vivo* mutagenicity testing, then registrants must submit a testing proposal for prior approval by ECHA. In vitro *OECD test guidelines include:* — OECD TG 471: Bacterial reverse mutation (Ames) test (27) — OECD TG 476: *In vitro* cell gene mutation test in mammalian cells (28) — OECD TG 473: *In vitro* chromosomal aberration test in mammalian cells (29) — OECD TG 487: *In vitro* mammalian cell micronucleus test (30) — OECD draft TG: *In vitro* mammalian cell gene mutation assays using the thymidine kinase gene (31) — OECD QSAR toolbox (26)

Table 1: continued

Acute toxicity
The studies do not generally need to be conducted, if the substance is classified as corrosive to the skin.
Registrants should use the 3T3 neutral red uptake cytotoxicity (32) test to predict non-toxic chemicals. Substances that have not shown oral acute toxicity up to a limit dose of 2,000mg/kg bodyweight no longer require testing for acute dermal toxicity as a second route of administration (33). If an *in vivo* test is perceived to be required, for example due to a positive result, the results of the 3T3 NRU test should be used to set starting doses (34) for an *in vivo* study using the most relevant route. Registrants should also use the OECD QSAR toolbox (26) to fill any data gaps and use results from the 3T3 NRU to support read-across.
Short-term aquatic toxicity
Testing does not need to be conducted, if the substance is highly insoluble in water or unlikely to cross biological barriers or if a long-term fish toxicity study is already available.
If testing is required, then: a) the fish embryo toxicity test (OECD TG 236 [35] and the EURL ECVAM recommendation [36]) should be used to predict fish acute toxicity as an alternative to the fish acute toxicity test (OECD TG 203); and b) registrants should use the OECD QSAR toolbox (26) to fill data gaps. As a last resort, the threshold approach (37) for acute fish toxicity testing should be considered.

The OECD website should be checked (38) for the most up-to-date versions of the documents. Other QSAR tools are available on the EURL ECVAM website (39).

This series features leading experts in the field of alternatives, including representatives from the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), REACH consultants, and industry. The first webinar focused on the use of Quantitative Structure–Activity Relationships (QSARs) and read-across, as these are critical to minimising testing for all endpoints. This event drew more than 800 registrants, and with a recording of the webinars available online, we expect to reach hundreds more. Additional webinars address specific Annex VII and VIII endpoints, including skin irritation and corrosion, serious eye damage and irritation, and in 2015 webinars (2) addressing skin sensitisation, acute oral toxicity, and acute toxicity to fish will be presented. This webinar series complements other avenues that PISC uses to advise registrants on minimising animal testing. For example, a recent *Chemical Watch* article (3) and poster (4), authored by PISC scientists, described how avoidable animal tests continue under REACH, and indexed current methods and strategies for minimising animal testing for the 2018 deadline (as described in Table 1).

Lobbying ECHA and the European Commission

To ensure that registrants test on animals only as a last resort, as required by the REACH regulation, it is essential that the relevant guidance and regulations be kept up-to-date with developments in alternative methods. PISC recently urged the European Commission (EC; 5) to ensure that the EU Test Method Regulation and REACH Annexes be updated expeditiously with the most current Organisation for Economic Co-operation and Development (OECD) test guidelines and methods recommended by the EURL ECVAM, and that requirements for obsolete animal tests be removed. These updates would avoid confusion over the use of adaptations to the standard data requirements outlined in the REACH annexes and therefore help avoid animal testing. PISC has been assured by the EC that it is considering several of its recommendations, and we expect significant updates to the REACH Annexes in the near future.

As an ECHA-accredited stakeholder since 2013, PISC has access to inter-governmental meetings relating to REACH, in order to encourage the replacement of testing on animals wherever possible. Further, PISC is an invited member of several Partner Expert Groups (PEGs), addressing updates to the endpoint-specific guidance. This level of participation allows us to ensure that updates to guidance incorporate all relevant advances in the Three Rs (*Replacement*, *Reduction* and *Refinement*) in relation to animal testing.

Ensuring the best use of existing data

Registrants are required to submit testing proposals to ECHA, prior to conducting tests for Annex IX and X endpoints (substances greater than 100 tonnes per annum). To ensure that the use of existing information has been optimised, third parties have 45 days to submit "scientifically valid information and studies that address the relevant substance and hazard endpoint, relating to the testing proposal". PISC reviews each proposal for new animal testing under REACH, and submits overlooked data from publicly available databases where relevant.

Intervention in precedent-setting appeal cases

Registrants can appeal decisions made by ECHA under the REACH regulation. PISC intervenes in cases that might be precedent-setting or relate to testing on large numbers of animals (for example, the case brought by CINIC Chemicals Europe Sàrl, which relates to reproductive toxicity testing that could use approximately 1,500 animals). By intervening in such cases, PISC ensures that the animal welfare implications of the appeal are considered at every stage of the appeal process.

European Ombudsman complaint against ECHA

In 2011, when ECHA published its first report on the use of alternatives to animal testing (6), it was immediately apparent that avoidable experiments on animals were taking place. For example, tests were conducted on animals in an attempt to assess the skin and eye irritation potential of chemicals, even after validated alternative methods had become available, and tests were conducted without the prior approval of a testing proposal. In 2012, the European Ombudsman launched an enquiry into the actions of ECHA (7), following a complaint submitted by PISC member PETA UK, alleging that ECHA was failing to ensure that alternative methods were being used wherever possible. In a landmark decision that could save millions of animals from suffering in laboratory experiments, the European Ombudsman agreed that ECHA's interpretation of its obligation was excessively restrictive and that ECHA was not fully applying its authority to minimise animal experiments, as required by law, and should begin to do so (8). The Ombudsman found that ECHA's refusal to ensure that dossiers comply with the principle of using animals only as a last resort is akin to informally amending REACH without involvement of the European Commission. The Ombudsman has issued clear direction for ECHA to request information from registrants to demonstrate compliance when required. Further, the Ombudsman's decision directs ECHA to inform Member States of all possible instances of non-compliance – not just proven violations.

An End to Testing Cosmetics on Animals in India

A major focus of PISC member PETA India has been to encourage the Indian government to follow the progressive examples of the EU, which has banned the testing of cosmetics and their ingredients on animals and the sale of animal-tested cosmetics, and Israel, which has banned testing on animals for both cosmetics and household products and the sale of those products, if they have been tested on animals elsewhere in the world. PETA India is the only animal rights organisation to hold an official seat on both Bureau of Indian Standards committees that set the precedents for testing the safety of cosmetics and household products: the Cosmetics Sectional Committee (PCD 19) and the Soaps and Other Surface Active Agents Sectional Committee (CHD 25), respectively. PETA India worked co-operatively with national regulatory agencies to promote the use of validated non-animal research methods and to reform government testing regulations. In addition, PISC provided expert advice on available non-animal methods leading to the replacement of three animal tests — skin sensitisation with guinea-pigs, and the acute oral toxicity limit test and the oral mucosal irritation test on rats — that had been required for cosmetics testing. In May 2014, a ban on testing cosmetics on animals was announced, and was quickly followed with an import ban in October 2014. While industry bodies were particularly resistant to these changes, PISC provided expert advice to PCD and CHD on the value of keeping these bans in place. In recognition of PETA India's work to change government policies regarding animal testing and its efforts to help consumers choose cruelty-free products, the international cosmetics company Lush awarded PETA India its 2012 prize for excellence in lobbying.

International Harmonisation

PISC and its members recognise that regulatory acceptance of non-animal techniques in one region or country is an open door to international harmonisation and the wider statutory elimination of animal testing methods. PISC members work extensively on the international testing guidelines that determine the exact nature and number of animals used in globally-standardised toxicity tests, which are used by companies complying with regulatory requirements for chemical safety testing.

PISC and its members address harmonisation on several fronts. Through the International Council on Animal Protection in OECD Programmes (ICAPO), PISC ensures that the best possible science on *replacement* strategies (as well as *reduction* and *refinement* strategies) are integrated into OECD guidelines on test methods and testing approaches. ICAPO has a voice at the OECD because PISC member PETA US recognised the OECD's importance in animal testing matters 15 years ago, and helped create an international coalition of animal protection groups that was accepted by the OECD as a recognised non-governmental organisation in 2002. PISC and its members' OECD work includes participation in expert groups developing *in vitro* assays for endocrine active chemicals, a guidance document to minimise testing overall for

endocrine active chemicals, a framework to minimise fish testing for eco-toxicology, a test guideline that would halve the number of animals used in developmental toxicity testing, and a strategy for including metabolism in non-animal assays. ICAPO was awarded the Lush lobbying prize in 2013 for its successful work with the OECD, and is now considered a world leader in the promotion of non-animal methods, approaches and policies.

EURL ECVAM has a critical role in determining the scientific acceptance and regulatory use of non-animal testing methods within the EU and globally. PISC is a member of the EURL ECVAM stakeholder forum, and involvement in this forum provides us with the opportunity to influence the policy of this institution and contribute to the validation process of *in vitro* methods at key stages by commenting on EURL ECVAM recommendations on validated test methods.

Conclusion

PISC's multi-disciplinary team uses a varied approach to minimise testing on animals internationally. For example, PISC members have been addressing the animal testing implications of REACH since the programme was first announced and PISC is building on this important work. Through a comprehensive approach, which involves informing registrants about alternatives to testing on animals, ensuring the best use of existing data by commenting on testing proposals, intervening in precedent-setting cases involving ECHA, and engaging with ECHA and the European Commission, PISC hopes to ensure that animal testing for REACH is conducted only as a last resort.

PISC also provides its members with expert scientific advice, which is essential to support campaigns to end testing on animals. This advice was a critical part of the campaign to end the testing of cosmetics on animals in India, where PISC provided technical expertise on non-animal methods that can be used to assess the toxicity of cosmetics and their ingredients.

By incorporating these and other strategies to influence and harmonise national and international regulations and test guidelines, PISC helps advance 21st century science across the globe.

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