A Multi-faceted Approach to Achieving the Global Acceptance of Animal-free Research Methods

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Summary — In 2015, the PETA International Science Consortium Ltd. was awarded the Lush Training Prize for its broad approach to education and training on the effective use of human-relevant, non-animal research techniques. The prize was awarded for work that included hosting workshops and webinars, initiating in-person training sessions and developing educational resources. The Consortium works closely with industry and regulatory agencies to identify and overcome barriers to the validation and use of alternatives to animal testing, by using an approach that identifies, promotes and verifies the implementation of these methods. The Consortium's recent activities toward replacing tests on animals for nanomaterials, pesticides and medical devices, are described, as examples of projects with broad applicability aimed at large-scale regulatory change.

Key words: *animal testing, AOP, chemical testing, cosmetics testing, data requirements, harmonisation, ICAPO, nanomaterials testing, non-animal, OECD, pesticide testing, QSAR, REACH, risk assessment, Three Rs, validation, webinars.*

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Introduction

The PETA International Science Consortium Ltd. (the Consortium) was established in 2012 to advance the use of human-relevant animal-free research methods. The Consortium seeks to bridge the gap between the development of these methods and their subsequent regulatory acceptance, through the funding of research and validation efforts and the promotion of international regulatory awareness, and by encouraging the uptake of non-animal test methods by the regulatory community. The diverse expertise of its scientists enables the Consortium to take a multi-faceted approach to these projects by working with policymakers, scientists and regulators on a wide range of issues. This article describes some of the Consortium's recent activities, from funding the development of novel tools in toxicology and human risk assessment, to providing educational outreach, and demonstrates that collaboration with regulatory officials and private industry scientists can successfully result in the replacement of tests on animals.

Education and Training

In 2015, the Consortium was awarded the Lush Training Prize, which honours achievements in the dissemination of information on non-animal methods among commercial scientists, researchers and students. The Consortium was recognised for its broad approach to education and training, which includes hosting workshops and webinars, initiating in-person training sessions, and developing educational resources (1). The Consortium maximises its reach and accessibility by providing valuable information for scientists on its website, including a detailed list of validated alternative methods that are accepted by regulatory authorities, fact sheets on the use of non-animal methods, and specific information on the testing of chemicals, pesticides, nanomaterials and other substances (2).

Of primary interest to the Consortium is the European Union regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). This regulation requires registrants to supply an increasing amount of information on the human health and ecological effects of chemicals, as the annual manufacturing or importation volume increases. For some chemicals, this requirement may lead to the use of thousands of animals in new tests. To help registrants and regulators prepare for the registration deadlines, the Consortium worked in partnership with *Chemical Watch*, the leading European regulatory news and information service for the chemical industry, to host a free webinar series on the use of non-animal methods. The webinars were presented by leading experts in the field, including industry and REACH consultants, academics and regulators. Hundreds of scientists and

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regulators registered for each one. A wide range of topics were addressed, such as the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox and the application of read-across, as well as non-animal methods for assessing skin sensitisation, skin and eye irritation, and acute systemic toxicity. Of those surveyed, 80% stated that they had gained useful information on incorporating non-testing or non-animal methods into a testing strategy, demonstrating the effectiveness of the webinars in facilitating change.

By evaluating the outcomes of initiatives like these webinars, the Consortium is able to maximise the impact of subsequent programmes, tailoring them according to the feedback received. Building on the success of the first webinar series, the Consortium next presented *Alternative Approaches for Identifying Acute Systemic Toxicity: Moving From Research to Regulatory Testing*, a webinar series and a workshop that focused on strategies for replacing *in vivo* acute systemic toxicity tests (3). This was followed by the webinar series *Alternative Approaches for Acute Inhalation Toxicity to Address Global Regulatory and Non-regulatory Data Requirements,* and another information gathering session, at which international experts discussed their experiences of using alternative approaches and strategised on regulatory acceptance.

Publication in scientific journals is another key avenue for information sharing. For example, the Consortium's recent publication *Modern Affinity Reagents: Recombinant Antibodies and Aptamers* highlighted the advantages of non-animal affinity reagents, which eliminate not only the animal welfare concerns associated with their production, but also the growing concern about the lack of quality and reproducibility of animal-derived antibodies (4).

The Consortium is also a key member of the International Council for Animal Protection in OECD Programmes (ICAPO), which seeks to ensure the widest possible integration of alternative methods into OECD Test Guidelines. In a recent project, ICAPO collaborated with the Laboratory of Mathematical Chemistry in Bulgaria to offer QSAR Toolbox training courses to regulators at the US Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxics. The Consortium also collaborates with other groups to provide training, such as the Institute for In Vitro Sciences, Inc., whose courses promote effective implementation of non-animal strategies at a decision-making level.

Funding Research and Validation Studies for Promising Non-animal Tests

To date, the Consortium and its members have contributed more than €1,800,000 (or $2,000,000; with in-kind donations, this figure rises to $4,000,000) toward the improvement and implementation of promising non-animal research methods by targeting those methods that require further development or validation prior to regulatory acceptance. Projects include the advancement of QSAR models to estimate chemical carcinogenicity, and non-animal models that test substances for acute and sub-chronic toxicity, skin sensitisation, skin irritation and endocrine activity.

The impact of the funding is often augmented through commercial collaborations. In one case, the Consortium teamed up with the MatTek Corporation to award free 3-D human tissue models to selected researchers who had submitted promising proposals for the use of these tissues in their work to replace testing on animals. The Consortium also funds long-term research projects. For example, researchers were awarded $200,000 for the first phase of a multi-year project to develop and validate an *in vitro* system that uses human cells to recreate an air–lung interface, which can then be exposed to aerosolised substances, creating a physiologically-relevant model of inhalation toxicity. The study was launched after a Consortium-organised workshop (5), and has initially focused on the development of pulmonary fibrosis following the inhalation of multi-walled carbon nanotubes (MWCNTs) (6), a nanomaterial used worldwide in consumer products. Because of the limited understanding of the adverse health effects resulting from MWCNT inhalation, this research is both timely and important (7). In other instances, the Consortium recognises opportunities to validate existing non-animal methods for regulatory use in place of required animal tests. For example, the Consortium is co-organising a validation effort to demonstrate that *in vitro* human tissue models can replace the use of rabbits in vaginal irritation tests that are required in order to market personal lubricants in the USA. To this end, targeted *in vitro* testing with human *in vitro* tissue models will be carried out as necessary, following the analysis of existing *in vitro* and *in vivo* data collected from personal lubricant manufacturers. If the US Food and Drug Administration (FDA) confirms the validity of the *in vitro* tissue model method, its use will be permitted in place of the rabbit test.

In vitro assays are vital to improving our fundamental understanding of toxicity, as the data produced can be used to pinpoint specific toxicity mechanisms, and can be added to data-sharing platforms, such as the Adverse Outcome Pathway (AOP) Wiki (8). An AOP is a conceptual framework that organises existing knowledge concerning biologically plausible and empirically supported links between molecular-level perturbation of a biological system and an adverse outcome (9). The well-considered use of AOPs has the potential to reduce tests on animals through the prioritisation of mod-

ern test methods (10). The AOP-Wiki provides a platform for AOP development, and to further encourage new contributors, the Consortium has launched a 'data challenge' to award prize money to those whose contributions are judged to be the most promising (11). These contributions will bring AOPs closer to practical application and regulatory acceptance.

Overcoming Barriers to the Adoption of Humane Methods

Because validation alone is not sufficient to ensure that scientifically credible alternative test methods are used and accepted in place of animals in every country, the Consortium works closely with regulatory agencies to identify and overcome barriers to global acceptance of methods.

For instance, Consortium scientists are collaborating with regulators worldwide to replace the experiments on animals which are used to satisfy pesticide registration data requirements with currently available non-animal methods. While alternative methods for evaluating eye irritation are now allowed by the EPA Office of Pesticide Programs (OPP) for antimicrobial cleaning products, they are not being widely used by companies because of a lack of global regulatory acceptance, industry uncertainty over reviewer familiarity and concerns that the alternative test may predict a greater hazard than the corresponding *in vivo* test. In the publication, *Bridging the Gap Between Regulatory Acceptance and Industry Use of Non-animal Methods*, the Consortium presented actions that could be taken to overcome such obstacles (12). Actions included: working to increase international harmonisation; proactively publicising the EPA's alternatives policies; providing incentives to companies that use the non-animal methods; and enhancing the training of regulatory reviewers.

Scientists from Consortium member PETA US also participate in a stakeholder group that is advising the OPP in its efforts to compare historical *in vivo* and *in vitro* skin and eye toxicity data, in order to evaluate the performance of non-animal methods with the pesticide class of chemicals. Additionally, with encouragement from PETA US, the OPP is exploring a transition from its current classification and labelling system to the Globally Harmonised System (GHS), a single worldwide system for classifying and communicating the hazardous properties of chemicals. As most *in vitro* methods have been designed to predict GHS hazard classes, the GHS provides the infrastructure for a consistent approach to the classification of chemicals (13).

Many regulatory authorities, not familiar with the use and scope of more recently validated alternative methods, retain requirements to use traditional animal tests. The acceptance of non-animal techniques by regulatory agencies in one region or country is an open door to international harmonisation and the wider statutory elimination of animal methods. For example, the success of PETA US in urging regulators to review and revise their regulations has led to the removal of the one-year dog toxicity study as a pesticide data requirement in the USA, the EU and Canada, and has provided a model for other countries, such as Brazil, to remove or reconsider the requirement as well (14). In India, Consortium members have prompted the implementation of strategies for reducing the number of animals used in pesticide testing (15). India's Recommendation Committee (RC) has agreed to allow some tests to be combined instead of being conducted separately, which can significantly reduce the number of animals used. Furthermore, the RC will allow the waivering of some tests on animals, such as those in which dogs are repeatedly fed pesticides for three months, if acceptable existing data can be provided.

Conclusion

Since its inception three years ago, the Consortium has achieved numerous successes in advancing the awareness, understanding and use of non-animal testing methods. It has used a comprehensive approach and diverse strategies for the harmonisation of national and international regulation and test guidelines, and has provided funding opportunities to help advance 21st century science around the globe. As a result, the Consortium has saved countless animal lives, whilst developing the relationships necessary to expand the knowledge and global acceptance of the most innovative animal-free techniques.

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