National Institute for Public Health
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RIVM 3R's *Quarterly*

February 2018

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RIVM 3R's Quarterly informs you on news and developments on 3R methods and animal-free innovations for the safety assessment of chemicals and medicines, including vaccines.

Chemicals

ECHA report on non-animal approaches

In November 2017, the European Chemicals Agency (ECHA) published a report on Non-animal approaches – Current status of regulatory applicability under the REACH, CLP and Biocidal Products regulations.
This report describes how currently available non-animal approaches can be used under the REACH Regulation and the Biocidal Products Regulation (BPR) to fulfil the information requirements and to reflect the needs of the Classification, Labelling and Packaging (CLP) Regulation.
In this report, the focus is on non-animal approaches that can be used to directly replace animal test methods. An overview is provided of available methods to reduce or refine animal testing, when no alternative is available. An important conclusion is that the full replacement of current toxicity tests by non-animal approaches is not yet possible.
Most progress is made in the area of animal tests required to test lower tier endpoints, such as skin corrosion/irritation, serious eye damage/eye irritation, skin sensitisation. For the more complex systemic endpoints, e.g. repeated dose toxicity and reproductive toxicity, progress is limited. ECHA mentions that methods such as grouping and read-across with existing test data to predict toxicity of substances may reduce the need for new tests on animals.
Many promising new approaches are developed. However, further standardization and validation as well as a continuous dialogue between researchers and regulatory authorities is required to ensure that innovations in non-animal approaches can be considered for regulatory use in chemical safety assessment.
To facilitate the identification of gaps and research needs, ECHA recommends to establish an inventory of non-animal approaches at different stages of development and regulatory applicability.
The full report is available at:
https://echa.europa.eu/-/more-progress-needed-to-replace-animal-tests-under-eu-chemicals-laws

Publication: round robin study to evaluate the reconstructed human epidermis (RhE) model as an *in vitro* skin irritation test for detection of irritant activity in medical device extracts

Together with 23 organizations RIVM published an article in a dedicated special issue of Toxicology In Vitro (on a large international round robin study to evaluate the possibilities to use the reconstructed human epidermis for the testing of irritant capacity of medical device extracts.
The 23 participating organizations cover all stakeholders involved in the safety evaluation of medical devices such as medical device companies, contract research organizations, universities, governmental institutes, and the producers of the epidermis model.
Assessment of skin irritation is an essential component of the safety evaluation of medical devices and is currently done in animal tests. OECD Test Guideline 439 describes the use of reconstructed human epidermis (RhE) as an *in vitro* test system for classification of skin irritation by neat chemicals. The round robin study was done to assess if this *in vitro* test method is applicable to medical devices as well.
Our results indicate that RhE tissue models can detect the presence of strong skin irritants at low levels in dilute medical device polymer extracts. Therefore, these models may be suitable replacements for the rabbit skin irritation test to support the biological evaluation of medical devices.
The full article can be downloaded at:
https://doi.org/10.1016/j.tiv.2018.01.001

Chemicals

JRC EURL ECVAM news

In November, EURL ECVAM organized the annual meeting of EURL ECVAM's regulatory network (PARERE) followed by a joined meeting of PARERE and the ECVAM stakeholder forum (ESTAF). The PARERE expert network is dedicated to the assessment of regulatory relevance of test methods proposed for validation. RIVM is representing The Netherlands in this network and one of our experts was present at this meeting. Updates were given of submissions for validation studies, the current activities for the toxicokinetics strategy were presented and discussed. In addition, the Horizon 2020 funded EUToxRisk project was presented and PARERE's input on the potential regulatory use of the results of this project was discussed. At the PARERE-ESTAF meeting the theme was: knowledge sharing. This was discussed around three areas: research, education and regulation.
https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvams-stakeholder-forum-and-regulatory-advisors-share-opinions-on-better-knowledge-sharing

EURL ECVAM published their annual Status Report in December 2017. This report provides an update on the development, validation, dissemination and regulatory acceptance of alternative approaches. The full report can be found here: *https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-status-reports*

Publication: expert opinions on the acceptance of alternative methods in food safety evaluations

Researchers at RIKILT, Wageningen University and Research, interviewed experts to formulate recommendations to increase acceptance of non-animal methods for kinetics, in particular in food safety testing. The experts agreed that the main driver for acceptance of non-animal methods was the ability of 3R methods to provide more mechanistic information. The main barriers were (i) uncertain predictability of 3R methods and lack of validation; (ii) insufficient guidance for regulators and industry; and (iii) insufficient harmonization of legislation. The experts were key stakeholders involved in food safety evaluations and gave their opinions on the most relevant factors that influence the acceptance and use of 3R methods. Recommendations given by the experts include the steering of regulatory data requirements as well as creating (funding) opportunities for development and validation of alternative methods for kinetics and development of guidances. The full article can be found here: *https://www.sciencedirect.com/science/article/pii/S0273230017303720*

New EU project PATROLS

RIVM is one of the partners in a new EU project (DG Research and Innovation) called PATROLS. The aim of the PATROLS project is to establish and standardize hazard assessment tools that accurately predict adverse effects caused by long-term low dose engineered nanomaterials (ENM) exposure. PATROLS focuses on both human and environmental systems to support regulatory risk decision making. The project is coordinated by Prof Shareen Doak of Swansea University, UK and involves 26 partners from Europe as well as Canada, Japan, Korea and the US. The contribution of RIVM is related to the assessments of biodistribution, *in vitro* advanced lung models, extrapolation of *in vitro* data to *in vivo* effects and dissemination of the projects results to stakeholders, ISO and OECD. PATROLS aims to deliver: 1) more realistic and predictive *in vitro* three dimensional (3D) lung, gastrointestinal tract (GIT) and liver models for mechanism-based hazard assessment; 2) cross-species models linking human and environmental systems; 3) innovative methods for sub-lethal hazard endpoints in ecologically relevant test systems and organisms, selected according to their position in the food chain; 4) robust *in silico* methods for exposure and dosimetry modelling, as well as hazard prediction. PATROLS aims to significantly improve nanomaterial hazard evaluation by developing advanced and reliable *in vitro* and *in silico* tools, thereby contributing to effective knowledge-based testing strategies using a weight of evidence approach. This will provide a sustainable solution to the considerable long-term challenges facing ENM hazard assessment, minimizing animal testing in support of the 3Rs and providing for self-regulation in industry. For more information see: *http://www.swansea.ac.uk/PATROLS*

Medicines

Workshop Report - A question-based method to validate animal models of disease

On January 30th, 2018 Utrecht University (UU) held a workshop on a question-based method to validate animal models of disease. The workshop is part of a project commissioned by the Dutch Ministry of Agriculture. It was a well-attended meeting chaired by Prof Dr Huub Schellekens (UU), with representatives from academia, government, industry and the Dutch regulatory agency. Prof Dr Schellekens opened the workshop by discussing the current issues with preclinical research. Dr Peter van Meer (CBG/UU) discussed current challenges and opportunities in establishing the preclinical proof-of-concept and Erik Doevendans (UU) showed that most EMA approved orphan drugs had relevant animal models and well-correlated endpoints. Guilherme Ferreira (UU) showcased a new tool to validate animal models of disease, the Question-Based Validation Sheet (QBVS), which was the focus of the workshop. The definition of validation was an important discussion topic, which was understood either as a statement of the complete simulation of the human condition or as an assessment of how well a model simulates each aspect of the human condition. The validation of models of disease using the QVBS was largely considered to be objective, although some margin of subjectivity remained. Overall, a group of major stakeholders contributed to a lively discussion on the many aspects of the use and implementation of the QBVS. The feedback from the audience made clear that despite some challenges, there is a need for the QVBS to help assess, validate and compare animal models. Future stakeholder engagements will focus on training sessions with scientists on the QBVS.

Vaccines

Removal from the European Pharmacopoeia of the *in vivo* mouse histamine sensitization test (HIST) for residual pertussis toxin on the final product

Over the years, there have been many initiatives to develop an animal-free alternative to the histamine sensitization test (HIST). The HIST is an *in vivo* mouse model that is used to detect residual pertussis toxin in the acellular pertussis (aP) vaccine, a vaccine that is used in Western countries against whooping cough (pertussis). A proposed alternative model for the HIST is the CHO cell clustering assay, but experts doubt the value of this assay for detecting pertussis toxin in the final product. Therefore the inclusion of the CHO cell clustering assay as an alternative for the HIST in the European Pharmacopoeia (the EU reference work for the quality control of medicines) was not to be considered relevant. In a recent inventory among government control laboratories it was now concluded that the scientific basis for the HIST is limited and that there are in fact no problems with residual pertussis toxin in these vaccines. Therefore, the Group of Experts Human Vaccines and Sera of the European Pharmacopoeia proposed to remove testing for residual pertussis toxin on the final product from the European Pharmacopoeia, by HIST or any alternative assay. The European national control laboratories are awaiting publication to remove the HIST from their own guidelines. This will result in reduction of animal testing for this vaccine.

Other news and developments

Dutch roadmap towards animal-free regulatory safety testing

In December 2017, RIVM finalized the "Roadmap towards animal-free regulatory safety assessment", as part of the project "Transition to animal-free innovations" commissioned by the Dutch Ministry of Agriculture. The roadmap describes activities needed for the transition towards safety assessment of chemical substances and medicines based on animal-free innovations, thereby maintaining the same safety level for humans, animals, and/or the ecosystem. The activities are established during workshops held with Dutch representatives of governmental institutes, regulatory authorities, academia, NGO's, and industry concerned with regulatory safety assessment. It was recognized that the transition takes place in a complex international regulatory field and that it should be focused at a better prediction of safety through non-animal innovations. This requires a substantial investment in commitment, coordination, continuity, communication, cooperation and costs (funding). Contributions can already be made to activities towards (1) development of a conceptual framework for safety assessment based on human physiology and biology; (2) development of "safe harbour" initiatives for products that need authorization before marketing, such as medicines and plant protection products; (3) creation of an information portal that combines existing information on animal-free innovations in a uniform way; and (4) creation of platforms and networks at regional, national, and international level to disseminate knowledge on animal-free innovations between different disciplines and to add to the information portal. The Dutch version of the Roadmap towards animal-free regulatory safety testing *can be found here*. An English version of the Roadmap will follow soon.

Strategic roadmap for establishing new approaches to evaluate the safety of chemicals and medical products in the United States

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) coordinated the development of the US strategic roadmap for establishing new approaches to evaluate the safety of chemicals and medical products. This roadmap is a resource to guide U.S. federal agencies and stakeholders seeking to adopt new approaches to safety and risk assessment of chemicals and medical products that improve human relevance and replace or reduce the use of animals. This document was developed with input from members of 16 federal agencies, multiple interagency workgroups, and input from the public. As such, it represents a consensus perspective that does not necessarily reflect opinions or policy of any specific agency or workgroup, and should not be taken as a commitment by any federal agency. The US strategic roadmap can be found here: *https://ntp.niehs.nih.gov/pubhealth/evalatm/natl-strategy/index.html*. Activities to implement the strategic roadmap goals are already underway.

Other news and developments

Collaboration RIVM and the Dutch Society for the Replacement of Animal Testing

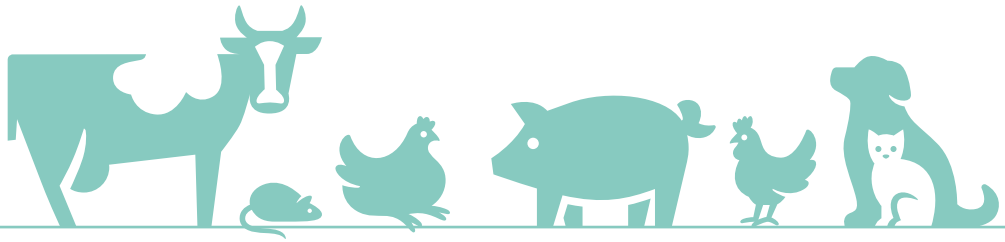
RIVM is working together with the Dutch Society for the Replacement of Animal Testing (dsRAT, or in Dutch "Stichting Proefdiervrij") to develop animal-free methods for the assessment of potentially toxic compounds, specifically for the developing embryo. In a PhD project, a testing method is further characterised and optimised that mimics parts of the early development of the embryonic brain. In this interview and video (both in Dutch), the project is briefly explained by the PhD student on the project, Victoria de Leeuw.

https://proefdiervrij.nl/phase/bescherming-babys-zonder-proefdieren/

https://www.rivm.nl/Onderwerpen/V/Vervangen_verminderen_en_verfijnen_van_dierproeven/3V_activiteiten_van_het_RIVM/Samenwerking_RIVM_en_Stichting_Proefdiervrij

Publication: Rethinking 3R strategies: Digging deeper into *AnimalTestInfo* promotes transparency in *in vivo* biomedical research

The German Federal Institute for Risk Assessment (BfR) and the German Centre for the Protection of Laboratory Animals (Bf3R) published an article on the analysis of nontechnical summaries (NTSs) of animal reseach studies in the *AnimalTestInfo* database. This database was developed in Germany to make the NTSs available in a searchable and easily accessible web-based format. The NTSs are publicly available documents provide an overview of the prospective uses of experimental animals. Dissemination of these documents through the *AnimalTestInfo* database enables the identification of areas in need of alternative strategies to help replace, reduce, and refine animal research. The researchers hope this data will inform governments and funding agencies in advancing the integrity and reporting of responsible animal research. The full article can be found here: *http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2003217*



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