National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

RIVM 3R's *Quarterly*

July 2018

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RIVM 3R's Quarterly informs you on news and developments of 3R methods and innovations for risk assessment of chemical substances and food, and for safety and efficacy assessment of medicines.

Chemical substances

Second BfR and RIVM workshop on animal-free innovations in safety assessment of chemicals

How can the process of validation, acceptance and use of animal-free innovative approaches to assess the safety of chemicals be facilitated? This was the topic of the second joint workshop of the German Federal Institute for Risk Assessment (BfR) and RIVM (13th and 14th of June 2018, Bilthoven). International experts from governmental institutes, regulatory agencies, industry, academia and animal welfare organisations discussed this topic in interactive sessions.

Validation is a procedure used to assess the reliability and relevance of a new test method. It is an important step towards regulatory acceptance, implementation and use of a new method for chemical safety assessment. Two scenarios can be followed towards an animal-free safety assessment framework. Firstly, an evolutionary scenario in which the current animal test-based system is modernised step by step as new animal-free approaches become available. Secondly, a revolutionary scenario that starts from scratch by describing the human biology and how this can potentially be affected by chemicals. Insight in the mechanisms involved in toxicity can subsequently be used to develop novel test methods. Ultimately, a framework combining different animal-free approaches covering human biology can be developed. The current validation procedures need to be adapted to be able to assess the reliability and relevance of such a revolutionary scenario.

During the workshop, a plenary session was followed by discussions in interactive breakout groups, enabling participants to exchange their point of view on how the process of validation, acceptance and use of these innovations of hazard and risk assessment in regulatory toxicology can be facilitated for these two scenarios. The topics of the breakout groups were: 1) Accelerating evolution: how to facilitate the regulatory acceptance of defined approaches and/or IATA; 2) Towards revolution: a new paradigm for risk assessment needs a new paradigm for validation; 3) Innovating validation: are case studies the answer?

Key recommendations were the need to define the biological relevance and uncertainties of new test methods and to use these aspects to evaluate their scientific validity. The need to increase experience and confidence was recognised. This can be achieved, for example, through case studies with defined sets of chemicals in which multiple stakeholders collaboratively participate. A workshop report will be published soon.

For years, BfR and RIVM have been dedicated to replace, reduce and refine animal testing for regulatory safety assessment. The Joint Declaration of Intent between both organisations has strengthened the collaboration in this area. This and future workshops aim to bring together all stakeholders to achieve acceptance, implementation and use of animal-free methods for safety assessment. More detailed information on the workshop can be found at:
https://www.rivm.nl/en/Documents_and_publications/Common_and_Present/Newsmessages/2018/Animal_free_innovations_in_safety_assessment_of_chemicals

Experts attending the joint BfR and RIVM workshop in Bilthoven

Chemical substances

OECD adopts new and updated test guidelines for chemical safety testing

In June 2018, the Organisation for Economic Co-operation and Development (OECD) adopted a number of new and updated test guidelines for chemical safety testing. These include updated animal-free test guidelines for eye irritation and skin sensitisation.

Revised Test Guideline 438 Isolated Chicken Eye Test method

Test guideline 438 Isolated Chicken Eye (ICE) Test method for Identifying Ocular Corrosives and Severe Irritants is revised to the ICE Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage. The revised ICE test method is based on an *in vitro* method that uses eyes collected from chickens obtained from slaughterhouses where they are killed for human consumption, thus eliminating the need for laboratory animals. It has been updated with histopathology of the cornea to increase the detection of certain types of chemicals inducing serious eye damage. The test guideline can be found at: *https://www.oecd-ilibrary.org/environment/test-no-438-isolated-chicken-eye-test-method-for-identifying-i-chemicals-inducing-serious-eye-damage-and-ii-chemicals-not-requiring-classification-for-eye-irritation-or-serious-eye-damage_9789264203860-en*

A publication of the International Association for Soaps Detergents and Maintenance Products (AISE) has – with support of the Netherlands - initiated the process of official adoption for the revised test guideline 438 in 2014. AISE states that the availability of the newly adopted revised guideline 438 is a major step forward to integration of robust and most effective *in vitro* test methods into the OECD framework. The test well complements the activities of the European Partnership for Alternative Approaches to Animal Testing (EPAA) for which AISE is a founding member.

A representative from the Dutch Authorities has commented: "We warmly welcome the *in vitro* test method that AISE has succeeded to realise. We applaud industries who are trying their very best to develop non-animal tests. The objective of the Netherlands is to accelerate the transition from working with animal testing to working with non-animal tests. The Netherlands are doing this via the 'Transition Programme for Innovation without the use of animals'. It would be great if the example set by AISE would be followed by many other industrial partners". The press release of AISE can be found at: *https://www.aise.eu/documents/document/20180628105550-oecdtest438ice_pressrelease_aise_28june2018_final.pdf*

Updated Test Guideline 442D In Vitro Skin Sensitisation

The existing test guideline 442D In Vitro Skin Sensitisation - ARE-Nrf2 Luciferase Test Method is updated by including the LuSens assay. This is a me-too assay of the Keratinosens™ assay. This test guideline addresses the second key event of the AOP for skin sensitisation: keratinocyte activation. The test guideline now covers two *in vitro* ARE-Nrf2 luciferase test methods: the KeratinoSens™ and LuSens. These assays measure the antioxidant/electrophile response-element (ARE) Nrf2 pathway, which is known to be activated by skin sensitizers.

The Netherlands contributed to several new and updated OECD Test Guidelines and Guidance Documents. All new and updated OECD test guidelines are available at the OECD website: *http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm*

Chemical substances

Publications on ontologies as a basis for reliable animal-free chemical hazard and risk assessment for humans

Given tremendous progress in biology, toxicology, and chemistry knowledge in recent decades, the time is right for serious consideration of options to move away from animal experimentation in chemical hazard and risk assessment. Individual alternative and animal-free assays as a replacement of individual animal studies have met with understandable reluctance in the scientific and regulatory arenas. An integrated conceptual approach based on mechanistic information built from all available information on biology, chemistry, and mechanisms of toxicity, might allow sufficient coverage of the biological system to provide the basis for reliable animal-free chemical hazard and risk assessment for man. RIVM published an article in Applied *In vitro* Toxicology describing construction of an ontology, which can be considered a network of adverse outcome pathways, including feedback loops representing homeostasis. The network ultimately aims to allows quantitative hazard and risk assessment for man, avoiding animal experiments. Full-text article available at: *https://www.liebertpub.com/doi/abs/10.1089/aivt.2017.0019*

In a special issue of Toxicology and Applied Pharmacology RIVM published an article on the design and validation of an ontology-driven animal-free testing strategy specific for developmental neurotoxicity testing. Developmental neurotoxicity entails one of the most complex areas in toxicology. Animal studies provide only limited information as to human relevance. A multitude of alternative models have been developed over the years, providing insights into mechanisms of action. The manuscript gives an overview of fundamental processes in neural tube formation, brain development and neural specification. To define testing strategies for neurodevelopmental toxicity testing, the challenge is in mining modern biology, toxicology and chemical information to feed intelligent designs. Full-text article can be found at: *https://www.sciencedirect.com/science/article/pii/S0041008X18300917?via%3Dihub*

In the same special issue of Toxicology and applied Pharmacology a consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes is published to which the RIVM contributed. This consensus statement voices the agreement of scientific stakeholders from regulatory agencies, academia and industry that a new framework needs adopting for assessment of chemicals with the potential to disrupt brain development. The statement can be read at: *https://www.sciencedirect.com/science/article/pii/S0041008X18300437?via%3Dihub*

In line with the consensus statement, a report was published describing an approach to construct a developmental toxicity ontology. Such an ontology will facilitate computer-based prediction of substances likely to induce human developmental toxicity. The report is the result of a consensus working group consisting of international experts developing a plan to create an ontology for developmental toxicity that spans multiple levels of biological organization, to which the RIVM contributed. The report can be found at: *https://onlinelibrary.wiley.com/doi/abs/10.1002/bdr2.1189*

Publication: Is current risk assessment of non-genotoxic carcinogens protective?

Non-genotoxic carcinogens (NGTXCs) are chemicals that do not cause direct DNA damage but induce cancer via other mechanisms. RIVM investigated whether current risk assessment of NGTXCs, which is based on No-Observed-Adverse-Effect-Levels (NOAELs), is protective against cancer. To answer this question, Benchmark Dose Modelling was applied on carcinogenicity data of 44 known NGTXCs. This study led to two main conclusions. First, a NOAEL derived from a subchronic study is similar to a NOAEL based on cancer effects from a carcinogenicity study, supporting the current practice in REACH of allowing carcinogenicity assays only when specific concerns are met. Second, both the subchronic and cancer NOAELs are, on average, associated with a cancer risk of around 1% in rodents. This implies that for those chemicals that are potentially carcinogenic in humans, current risk assessment of NGTXCs may not be completely protective against cancer. These results call for a broader discussion within the scientific community, followed by discussions among risk assessors, policy makers, and other stakeholders as to whether or not the potential cancer risk levels that appear to be associated with currently derived health-based guidance values (HBGVs) of NGXTCs are acceptable. The manuscript has been published in Critical Reviews in Toxicology: *https://www.tandfonline.com/doi/full/10.1080/10408444.2018.1458818*

Chemical substances

EPAA project on alternative approach for cancer risk assessment

Environmental chemicals are subjected to extensive human health risk assessments before being marketed. Currently, cancer risk assessment of agrochemicals still relies on animal-demanding 2-year rodent cancer bioassays. In the context of a project funded by the European Partnership for Alternative Approaches to Animal Testing (EPAA), RIVM aims to develop an alternative, mechanism-based approach for the assessment of non-genotoxic carcinogenic agrochemicals. This may lead to a substantial reduction in the number of rodent cancer bioassays, and thus in the number of laboratory animals, required for cancer risk assessment. More information can be found at: *https://ec.europa.eu/growth/sectors/chemicals/epaa_en* and *file:///R:/Projecten/E132524%20EPAA/Literatuur/Annual%20report%202017_web.pdf*

EURL ECVAM news on chemical mixture safety assessment

The Joint Research Centre (JRC) has investigated recent scientific progress on the combination effects of chemicals and how to address safety for humans and environment. The issues and specific challenges are described in a policy brief (see *ec.europa.eu/jrc/something-nothing-ensuring-safety-chemical-mixtures*). JRC collaborates with different research consortia that focus on chemical mixture safety assessment, including *Euromix*, that is coordinated by the RIVM and *HBM4EU* in which RIVM is a involved as well. More information can be found at: *https://ec.europa.eu/jrc/en/news/chemical-mixtures-safety*

Medicines

Publication: recommendations of the VAC2VAC workshop on the design of multi-centre validation studies

Within the Innovative Medicines Initiative 2 (IMI 2) project VAC2VAC (Vaccine batch to vaccine batch comparison by consistency testing), RIVM contributed to a workshop that has been organised on optimising validation studies for non-animal methods for vaccine testing. During the workshop, aspects of validation within the consistency approach context were addressed. This workshop report summarises the discussions and outlines the conclusions and recommendations agreed on by the workshop participants. These include recommendations to encourage manufacturers to play a more active role by identifying suitable non-animal methods, providing relevant samples, or sponsoring studies. For multi-centre studies, availability of sufficient resources for validation and implementation of new methods is crucial and the availability of critical reagents and reference preparations should be secured. A number of the recommendations are addressed to the VAC2VAC project itself and will be followed up by the partners.
The workshop recommendations can be found at: *https://www.sciencedirect.com/science/article/pii/S1045105618300150?via%3Dihub* and *https://ec.europa.eu/jrc/en/science-update/validation-non-animal-methods-vaccine-testing*

Other news and developments

Scientific Advisory Committee of EURL-ECVAM renewed

In April this year, the EURL ECVAM Scientific Advisory Committee (ESAC) was appointed. Prof. Dr. Aldert Piersma of the RIVM is again one of the members of ESAC (*http://ec.europa.eu/regexpert*). ESAC advises EURL ECVAM on scientific and technical issues related to non-animal test methods and approaches. This year ESAC will assess the scientific validity of alternative methods to assess chemicals for their skin sensitisation potential. Antibodies and new generation affinity reagents produced using animal-free technologies will be evaluated as well.
https://ec.europa.eu/jrc/en/science-update/eurl-ecvam-renews-its-scientific-advisory-committee

Report on Innovative 3Rs from the EU-ANSA Research Cluster

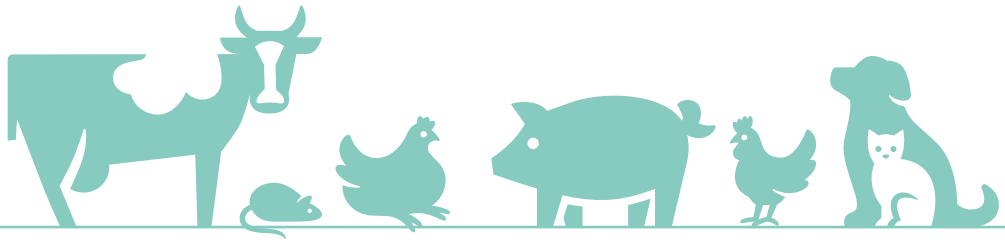
On 24 April 2018 the EU Agencies Network on Scientific Advice (EU-ANSA) Research Cluster published a report called 'Innovative 3Rs (Replacement, Reduction and Refinement of animal testing) approaches for the prediction of properties of chemicals, cosmetic ingredients, medicines, environmental contaminants and other regulated products'. Several EU-ANSA agencies involved in safety assessment of chemicals and products containing chemicals are interested in communicating R&D needs for better non-animal tests and approaches.
The report was prepared by the European Chemical Agency (ECHA), the European Medicine Agency (EMA), the Scientific Committee for Consumer Safety (SCCS), the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) in consultation with the Institute for Health and Consumer Protection of the Joint Research Centre.
An Introductory is available at: *www.emcdda.europa.eu/Introductory_text_for_3Rs_reseach_cluster*. The full report is available at: *www.emcdda.europa.eu/ANSA_Research_Cluster_Innovative_3R_approaches*

Dutch roadmap towards animal-free regulatory safety testing

The English version of the Dutch roadmap towards animal-free regulatory safety testing has been published recently: *https://www.rivm.nl/dsresource?objectid=571f1221-8ada-4488-99ef-2424acd50d30&type=pdf&disposition=inline*.

Inventory of 3Rs Knowledge sources

In 2016, EURL ECVAM published a study where they compiled knowledge sources (websites, publications, databases, organisations, events, expert groups, etc.) relevant to the 3Rs: Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing. Based on this inventory, the company Douglas Connect has developed a user friendly online application, DC 3Rs, that allows the user to consult 3Rs knowledge sources listed in the EURL ECVAM study. All available resources can be easily browsed, filtered and visualised in real-time. The EURL ECVAM study can be found at: *http://data.jrc.ec.europa.eu/dataset/jrc-eurl-ecvam-eurl-ecvam-3rs* and *https://publications.europa.eu/en/publication-detail/-/publication/58919142-f1b8-11e6-8a35-01aa75ed71a1/language-en*, the DC 3Rs app can be found at: *https://3rs.douglasconnect.com/*.



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