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

RIVM 3R's *Quarterly*

October 2017

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RIVM 3R's Quarterly informs you on news and developments in the area of 3R methods that can be used for risk assessment of chemical substances, food, and safety and efficacy assessment of pharmaceuticals, including vaccines.

Chemical substances

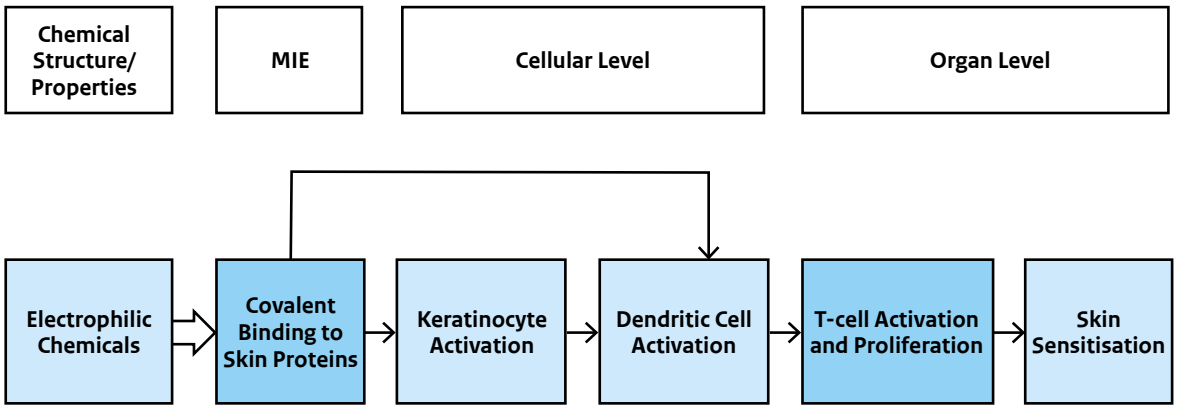
Fall meeting of the Risk Assessment section of the Dutch Society of Toxicology

In October the Risk Assessment section of the Dutch Society of Toxicology organized the meeting "Making sense of sensitisation" at Charles River in Den Bosch. The focus was on the state of affairs with regard to the implementation of non-animal test methods for skin sensitisation. The talks focused on the currently available OECD test guidelines, the most important features of these tests and the guidance on how to use them in defined and integrated approaches to testing and assessment. The experience so far from labs that have implemented these assays was presented by industry. Speakers were Janine Ezendam from RIVM, Walter Westerink from Charles River, and David Hart from AkzoNobel. RIVM presented on the regulatory need for non-animal approaches. The presentation focused on the development of OECD test guidelines and guidance on how to design a testing strategy to combine these methods for hazard identification and potency assessment. Charles River presented on non-animal skin sensitization testing under REACH. Fit-for-purpose validations of DPRA, ARE-Nrf2 Luciferase (KeratinoSensTM, LuSens) and U-SENS using appropriate proficiency chemicals were presented. Moreover, a tiered testing strategy using non-animal testing for mono-constituents, multi-constituents and UVCBs was presented and discussed. AkzoNobel presented on *in vitro* skin sensitisation testing and its benefits and limitations, sharing industry experience with these testing methods. A report from the meeting including presentations will be available soon at the website of the Dutch Society for Toxicology at *https://toxicologie.nl/en/category/sections/risk-assessment/*.

Revision OECD Test Guidelines for *in vitro* skin sensitisation

Several OECD "Test Guidelines for Testing of Chemicals in Section 4 on Health Hazards" have been recently revised. TG442 on *in vitro* skin sensitisation is revised to include two novel *in vitro* assays that address key event 3 of the skin sensitisation adverse outcome pathway (AOP): keratinocyte activation. The AOP for skin sensitisation is depicted in **Figure 1** and describes the key events that lead to skin sensitisation, taken from the AOP wiki (*https://aopwiki.org/wiki/index.php/Aop:40*). This test guideline now provides three *in vitro* test methods: the human cell line activation test (h-CLAT), the U937 Cell Line Activation Test (U-SENS) and the Interleukin-8 Reporter Gene Assay (IL-8 Luc assay). These *in vitro* assays can be used to discriminate skin sensitisers from non-sensitisers. For regulatory purposes, these test methods cannot be used as single methods; they need to be combined with test methods that address other key events of the AOP. Furthermore, these assays cannot be used on their own for potency assessment. Combining quantitative read-outs from different skin sensitisation assays in defined approaches may contribute to the assessment of potency The TG is available at: *http://www.oecd-ilibrary.org/environment/test-no-442e-in-vitro-skin-sensitisation_9789264264359-en*
OECD released several new or revised test guidelines in October 2017. More information available at: *http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm*

Figure 1. *AOP Covalent Protein binding leading to Skin Sensitisation. The molecular initiating event (MIE) is covalent binding of the chemical to skin proteins. At the cellular level, two key events (KE) are triggered by this MIE: KE2, which is keratinocyte activation and KE3, which is dendritic cell activation. Together these events lead to KE4 at the organ level, lymphocyte activation and proliferaton, resulting in the adverse outcome, skin sensitisation.*



Chemical substances

Publication: AOP respiratory sensitisation

In September, a special issue of the journal Applied *in vitro* Toxicology was published on "Adverse Outcome Pathways as Versatile Tools in *In vitro* and In Silico Toxicology – Part I". Several papers are open access. RIVM contributed to the article "An Adverse Outcome Pathway for Sensitization of the Respiratory Tract by Low-Molecular-Weight Chemicals: Building Evidence to Support the Utility of *In vitro* and In Silico Methods in a Regulatory Context". This AOP was developed by a group of international experts in this field. The AOP was used as a framework to organize all available mechanistic information in literature and to identify key events. Knowledge gaps were identified and recommendations for future research were made. This AOP provides insight into predictive tests that may in combination support the hazard identification of respiratory sensitizing chemicals. The full article can be downloaded at: *http://online.liebertpub.com/doi/full/10.1089/aivt.2017.0010*

Opinion: Validation redefined

RIVM published an opinion on the needs and opportunities to redefine validation of alternative methods to animal testing. *In vitro* chemical hazard assessment is moving from individual assays to combinations of assays in batteries and testing strategies guided by adverse outcome pathways. This has consequences for the way individual assays and testing strategies are validated. The authors propose that quality criteria of reproducibility and transferability and description of the chemical applicability domain remain essential at the level of individual assays. Validation in terms of predictivity of individual assays based on a variety of chemicals is no more relevant. Rather, sufficient coverage of the biological domain studied by a battery of complementary assays should be the prime determinant of the validity of test batteries and testing strategies. The full article can be viewed at: *https://doi.org/10.1016/j.tiv.2017.10.013*

Publication: *In vitro* to *in vivo* extrapolation of effective dosimetry in developmental toxicity testing: Application of a generic PBK modelling approach

RIVM published an article on the incorporation of kinetics to quantitative *in vitro* to *in vivo* extrapolations (QIVIVE). This is a key step for the realization of a non-animal testing paradigm for regulatory toxicology. The use of Physiologically-Based Kinetic (PBK) modelling for determining systemic doses of chemicals at the target site is accepted to be an indispensable element for such purposes. Nonetheless, PBK models are usually designed for a single or a group of compounds and are considered demanding with respect to experimental data needed for model parameterization. Alternatively, we evaluate here the use of a more generic approach, i.e. the so-called IndusChemFate model, which is based on incorporated QSAR model parametrization. The model was used to simulate the *in vivo* kinetics of three diverse classes of developmental toxicants: triazoles, glycol ethers' alkoxyacetic acid metabolites and phthalate primary metabolites. The full article can be downloaded at: *https://doi.org/10.1016/j.taap.2017.07.021*

Report of an ECETOC workshop: Applying 'omics technologies in chemicals risk assessment

RIVM contributed to a workshop entitled "Applying 'omics technologies in chemicals risk assessment", organised by theEuropean Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), from 10 to 12 October 2016 in Madrid, Spain. Thirty-six invited experts from Europe, Canada, Japan, and the United States participated in the workshop. It was a multi-stakeholder group including representatives of the European Commission, OECD, national authorities, academia, and industry. Multi-expert teams drafted frameworks on best practices for (i) a Good-Laboratory Practice-like context for collecting, storing and curating 'omics data; (ii) the processing of 'omics data; and (iii) weight-of-evidence approaches for integrating 'omics data. The workshop participants confirmed the relevance of these Frameworks to facilitate the regulatory applicability and use of 'omics data, and the workshop discussions provided input for their further elaboration. Additionally, the key objective (iv) to establish approaches to connect 'omics perturbations to phenotypic alterations was addressed. Generally, it was considered promising to strive to link gene expression changes and pathway perturbations to the phenotype by mapping them to specific adverse outcome pathways. While further work is necessary before gene expression changes can be used to establish safe levels of substance exposure, the ECETOC workshop provided important incentives towards achieving this goal. The full workshop report can be found at: *https://doi.org/10.1016/j.yrtph.2017.09.002*

Medicines

ICH S5(R3) Draft Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals includes possibilities for alternative methods replacing animal testing

Since the adoption of the ICH[1] S5(R2) guideline in 1995, over 20 years of experience has been gained with the testing of pharmaceuticals using both current, and novel testing paradigms. Scientific, technological and regulatory knowledge has also evolved significantly since then. Consequently, there are now opportunities for modernising existing testing paradigms to enhance human risk assessment, while also potentially reducing animal use. As a result of a proposal of the EMA and the Dutch and Belgian regulatory agencies (CBG-MEB and FHMP), a revision of the ICH S5 guideline was initiated in 2015. In the current step 2 draft version of the ICH S5(R3) guideline, alternative test systems may replace animal testing under certain predefined conditions. For the first time, qualification criteria are provided in this guideline to enable the evaluation of (batteries of) alternative assay for developmental toxicity testing for use in risk assessment for regulatory purposes. In addition, a comprehensive list of reference compounds for qualifying alternative assays for developmental toxicity is provided, consisting of 50 known developmental toxic pharmaceuticals and 16 negative compounds. The ICH S5(R3) guideline is expected to be finalized late 2019. The ICH S5(R3) draft step 2 guideline and further information on this procedure can be found here: *http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5/S5_R3__Final_Concept_Paper_27Mar2015.pdf*

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S5/ICH_S5R3ExplanatorySlides_20171006.pdf

[1] The ICH (International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) aims to achieve greater harmonisation worldwide to ensure that safe, effective, and high quality medicines are developed and registered in the most resource-efficient manner. A list of regions participating in ICH, both regulatory and industry, can be found here: *http://www.ich.org/about/membership.html*

Other news and developments

Scientific discussion on non-animal methods in the House of Representatives of the Netherlands

In September the House of Representatives of the Netherlands organized a round table discussion on development of non-animal research methods. From RIVM, Aldert Piersma and Theo Vermeire contributed to discussions on scientific development and the implementation of non-animal test methods. All scientists agreed that the use of animal methods for scientific purposes should be reduced. One of the drivers mentioned for continuation of using animals in research is “experience”. Vice versa, in general, scientists are reluctant to rely on results from non-animal test systems, due to a lack of experience and confidence. This should change. There should be strong national and international leadership to stimulate and coordinate the development and implementation of non-animal methods. Stakeholders, including scientists, industry, and policy makers should collaborate to build experience and trust in those methods. These are the first steps that should be taken towards becoming a world leader in laboratory animal-free innovations, an ambition formulated by the Dutch Minister of Agriculture in 2016.

The opinion papers (in Dutch) of all invited scientists to the round table discussion can be found at: *https://www.tweedekamer.nl/debat_en_vergadering/commissievergaderingen/details?id=2017A02149*

A news item on the round table discussion (in Dutch) can be found at: *https://nos.nl/artikel/2192939-nederland-kan-met-wat-moeite-koploper-dierproefvrij-onderzoek-worden.html*

Other news and developments

Meeting local organizing committee World Congress on Alternatives and Animals used for life sciences Maastricht

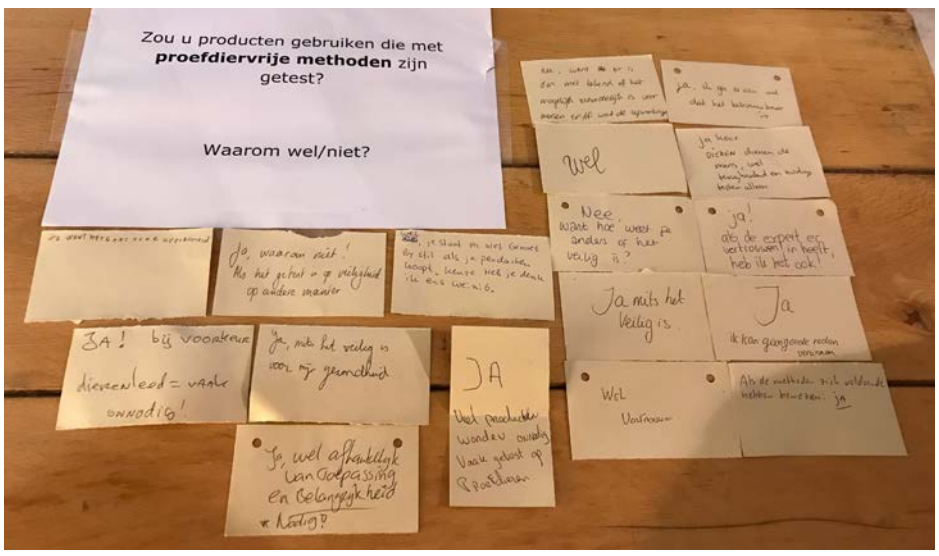
In October a meeting of the local organizing committee of the 11the World Congress on Alternatives and Animals used for life sciences (WC11) was organised. WC11 will take place on 23-27 August 2020 in Maastricht, The Netherlands and is hosted by Maastricht University. RIVM is involved in the organizing committee which includes experts from many different scientific disciplines from the Netherlands, Belgium and Germany. The meeting was dedicated to the scientific program, on communication and on sponsoring. Suggestions for topics can be submitted through our website: *http://www.wc11maastricht.org/*. On LinkedIn, Facebook and Twitter the committee provides updates and interacts with the scientific community. Links can be found on the website.

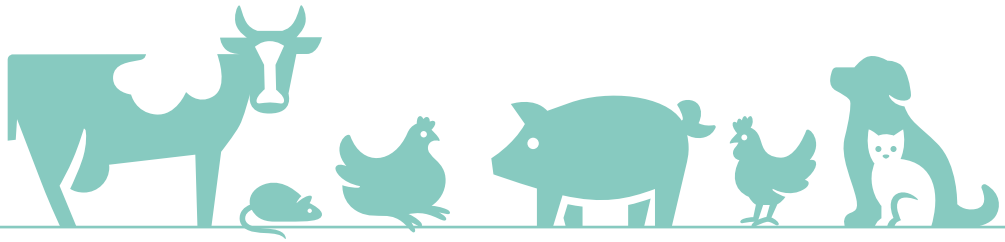
New Innovation Center for 3R methods in Belgium

In September the Free University in Brussels launched a new innovation center for 3R methods: *http://www.ic-3rs.org/*. This center aims at increasing the visibility of alternative methods by enhancing communication, building the local network and supporting the development of *in vitro* methodologies, throughout the Belgian region and Europe.

RIVM public dialogue on non-animal methods

During the Dutch Science Weekend, RIVM organised the "RIVM kennisparade". During this event, a variety of RIVM projects were presented to the public, with the aim to start a dialogue. Two of the RIVM projects on "non-animal testing" were also presented. These were the collaboration between RIVM and the Dutch foundation Stichting Proefdiervrij, and the RIVM activities on the roadmap towards animal-free regulatory safety testing. For the latter, the public was asked whether they would use products that are tested using non-animal testing systems. This led to interesting conversations and answers ranging from "yes, only if it is safe", to "no, because then you can't be sure about safety", and "yes, I trust the expert's opinion". A video on the collaboration of RIVM with Stichting Proefdiervrij can be found at: *https://twitter.com/rivm/status/916628409417175040*.





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