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

RIVM 3R's *Quarterly*

April 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 and safety and efficacy assessment of pharmaceuticals, including vaccines.

Chemical substances

EURL-ECVAM status update

Annually, EURL-ECVAM provides a status report that gives an update on the progress made in the development, validation and regulatory acceptance and use of alternative methods and approaches and their dissemination. The status report shows that validation studies of five *in vitro* test methods were evaluated by EURL ECVAM's Scientific Advisory Committee (ESAC). These include one test method that could be used for assessment of skin irritation, two test method for the prediction of serious eye damage and/or eye irritation potential and two test methods for skin sensitisation testing. This shows that progress is made only at the level of "simple" toxicological endpoints, whereas for the more complex toxicological endpoints no alternative test methods were validated last year.
Source: *http://publications.jrc.ec.europa.eu/repository/handle/JRC103522*.

New report of the National Academies of Sciences, Engineering and Medicine (NAS)

The US National Academies of Sciences, Engineering and Medicine published a report entitled: *Using 21st Century Science to Improve Risk-Related Evaluations.* This report is a follow-up of the National Research Council (NRC) report *Toxicity Testing in the 21st Century: a Vision and a Strategy* that provides a vision on toxicity testing relying on *in vitro* high-throughput test methods and computational models, rather than animal testing. In the NRC report *Exposure Science in the 21st Century: a Vision and a Strategy* a vision on exposure science and assessment is provided. The most recent NAS report provides recommendations on how to incorporate emerging science and technologies in the field of toxicity testing and exposure assessment into risk-based evaluations. The report identifies promising areas that could benefit from incorporating 21st century science and illustrates this with a number of case studies. Challenges are described as well in this report, including the issue that current validation procedures are not fit to test the high amount of assays and models that emerge from the Tox21 and exposure science research projects. The report concludes that despite the many challenges, 21st century science holds great promise for advancing risk assessment and ultimately improving public health and the environment.
Source: *http://dels.nas.edu/Report/Using-21st-Century-Science-Improve/24635*

ECHA advice on using non-animal test methods for REACH

In last years, regulatory accepted non-animal test methods that provide information on skin and eye irritation and corrosion and skin sensitisation became available. In 2016 the European Chemicals Agency (ECHA) revised the information requirements described in the REACH Annexes, which made non-animal testing the default test methods to obtain information on skin corrosion and irritation, serious eye damage and eye irritation and skin sensitisation. For these endpoints, justification is required if the animal test is conducted, which is only possible if the non-animal test methods are not suitable for testing a specific substance or cannot be used for classification and risk assessment. In addition, ECHA revised the guidance to reduce acute toxicity tests in rodents, by adding the possibility to waive this test using a weight-of-evidence approach. Recently, ECHA's guidance has been updated to give advice on when and how to use these non-animal test methods for REACH purposes.
Source: *echa.europa.eu/new-advice-on-using-non-animal-test-methods*

Validation redefined?

This question was addressed during a joint workshop of BfR and RIVM which was held in Berlin on March 23rd/24th. International experts from government, regulatory agencies, academia and industry discussed validation and regulatory acceptance of alternative methods with an emphasis on innovative testing strategies.
Current validation procedures were developed for individual alternative test methods. These procedures are essential in the process of regulatory acceptance and implementation in legal frameworks for chemical substances. Since a single alternative test method usually cannot replace an *in vivo* test method, computational and *in vitro* tools are combined in testing strategies, covering our mechanistic understanding of the toxicology of interest. There is no procedure how to validate testing strategies and this hinders regulatory acceptance and use. In the multi-stakeholder BfR-RIVM workshop, international experts from regulatory authorities, test methods developers and users from academia and industry, as well as experts participating in the OECD Test Guidelines Programme participated. The workshop aimed at defining a strategy to facilitate a more effective process of validation and regulatory acceptance of alternative testing strategies. During the workshop a mechanism-driven approach for the validation of testing strategies was discussed, as well as drivers and barriers in the process of validation and regulatory acceptance. The results of the workshop will be published soon.

Medicines

RIVM, in collaboration with the Dutch Medicines Evaluation Board and the Health and Environmental Safety Institute (ILSI-HESI), has produced a series of publications on database analyses of existing animal studies on carcinogenicity and developmental toxicity of pharmaceutical compounds. The aim was to retrospectively analyze existing data with a view to design scenarios for reduced animal testing in the future. In developmental toxicology, usually rat and rabbit studies are performed, with the most sensitive species often determining risk assessment outcomes. The analysis indicated that in around 70% of cases both species showed similar sensitivity, indicating that a second species had not given essential additional information. In the remaining comparisons either rat or rabbit was more sensitive. These findings are currently discussed in the European Medicines Agency with a view to change test guidance reducing animal studies. For carcinogenesis, alternative approaches to the 2-year carcinogenicity study have been explored. Recent studies demonstrate the added value of using risk factors from chronic (i.e. 6-month) toxicity studies as negative predictors. Results from the retrospective analyses performed show that this approach may also be applicable when using information from sub-chronic (3-month) toxicity studies. Furthermore, the approach can be improved by taking into account pharmacological properties.

The corresponding publications can be found here:
https://www.ncbi.nlm.nih.gov/pubmed/27848393
https://www.ncbi.nlm.nih.gov/pubmed/27766926
https://www.ncbi.nlm.nih.gov/pubmed/27614137
https://www.ncbi.nlm.nih.gov/pubmed/27790617

Scientific Committee European Commission publishes a preliminary opinion on the need for non-human primates in biomedical research, production and testing of products and devices

Following a request from the European Commission, its Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) has updated a former opinion on 'The need for non-human primates in biomedical research, production and testing of products and devices'. The preliminary opinion is currently under public consultation and written comments can be made until 26 March of 2017.

SCHEER reviewed new evidence from literature to update their former opinion. The most important conclusion is that based on the current state of knowledge it is not possible to propose a timetable for phasing-out the use of non-human primates in Europe. Recommendations are porvided on how to advance 3Rs for the use of non-human primates, including alternative methods, training, improvement of techniques and protocols, sharing of knowledge and removal of barriers and research needs.
Source: *ec.europa.eu/scientific_committees/scheer_consultation*

Vaccins

Dissertation: Cell cultures may require fewer laboratory animal experiments for testing vaccines

Dutch government policy is to reduce the use of animal experiments in biomedical research. In her dissertation, Marieke Hoonakker proved that cell cultures can be used as part of the quality control process for vaccines, at least in the case of whooping cough vaccines. Her research is a step towards the reduction and replacement of animal experiments.

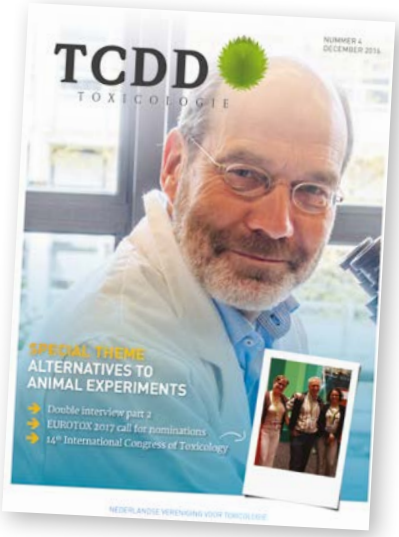
During their development, vaccines are exhaustively tested for their protective and potentially harmful effects. And once vaccines go into production, each batch is tested for effectiveness and safety. This usually requires the use of compulsory standardised animal experiments for vaccines such as diphtheria, tetanus and whooping cough. These animal experiments are controversial due to the wide range of variation in the test results and the discomfort for the laboratory animals. There are also immunological differences between the laboratory animals and humans.

Hoonakker studied whether cell cultures might be a possible alternative for these standardised animal experiments. To do so, she used whooping cough vaccines as a model and showed that the quality and safety of these vaccines can also be tested effectively using cell cultures. "The studies in my dissertation show that cell culture methods can differentiate between effective and less-effective whooping cough vaccines, and between safe vaccines and unsafe ones. Vaccination against whooping cough is still necessary, so it is vital that we can develop and validate these methods further in order to reduce the use of animal experiments in vaccine quality control."
The press release on the dissertation can be found on: *https://www.uu.nl/en/events/dissertation-cell-cultures-may-require-fewer-laboratory-animal-experiments-for-testing-vaccines*.

Other news and developments

Professorship "Substances and Prenatal Health Protection"

RIVM, the Institute for Risk Assessment Sciences (IRAS), Utrecht University, and the Dutch foundation Stichting Proefdiervrij have started a collaboration on the development of animal-free approaches to chemical hazard and risk assessment in the area of prenatal developmental toxicity. As a consequence of this collaboration, as of January 2017, the professorship of Prof. Dr. Aldert Piersma, reproductive toxicologist at RIVM, has been enhanced and renamed to "Substances and Prenatal Health Protection". The collaboration provides funding for a PhD student on the project and generates awareness about the relatively high experimental animal use in current developmental toxicity testing and the need for a transition to animal-free innovative methodologies. RIVM develops alternative methods using e.g. embryonic stem cells and computer modelling of embryo development. A recent double interview on the subject between Piersma and Marja Zuidgeest, director of Stichting Proefdiervrij, appeared in TCDD, the on-line journal of the Dutch Society of Toxicology.



The interview can be found on: *http://toxicologie.nl/images/TCDD/2016_TCDD_04_Double-Page_Spread.pdf*.

Other news and developments

European Commission

The EU Joint Research Centre (JRC) published a report to respond to the *European Citizens' Initiative "Stop Vivisection"* that was submitted in 2015 to the European Commission. It called for a new regulatory framework to replace Directive 2010/63/EU and to phase out all use of animal experiments. In its response, the EU explained that it is committed to animal welfare and it aims to meet this objective while striving to also protect human health and the environment. Despite significant progress in the development of alternative approaches, considerable scientific challenges remain for the more complex endpoints in basic and applied research, pharmaceutical product development and safety testing of substances. Thus, the complete replacement of animal studies is currently not possible while needing to ensure a high level of protection of human and animal health and the environment. Source: *ec.europa.eu/environment/chemicals/lab_animals/pdf/vivisection*.

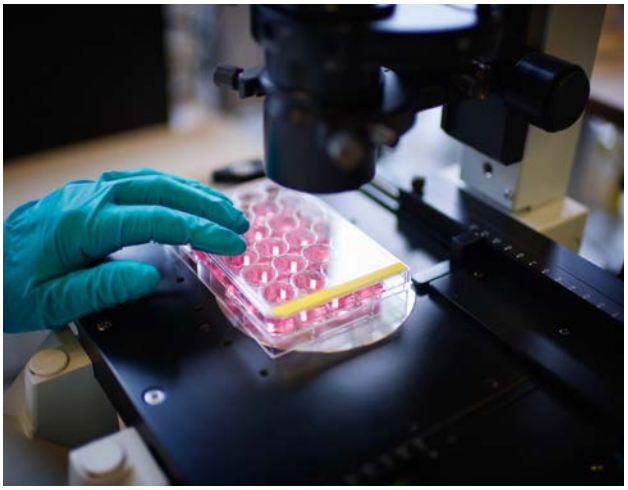
In order to accelerate the development and uptake of non-animal approaches in research and testing the Commission identified four actions:

1. Accelerating progress in the Three Rs through knowledge sharing
2. Development, validation and implementation of new alternative approaches
3. Enforcement of compliance with the Three Rs principle and alignment of relevant sector legislation
4. Engaging in a dialogue with the scientific community

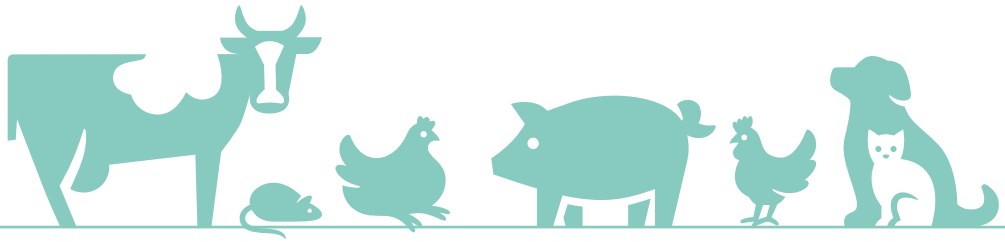
The recent report of the EU JRC reports on the first action, to assess the current situation regarding the sharing of knowledge which is relevant to the 3Rs.
The EU Joint Research Centre (JRC) carried out a study of available knowledge on the replacement, reduction and refinement (the 3Rs) of animal procedures used in research and testing. JRC mapped available knowledge by profiling over 800 knowledge sources relevant for the 3Rs and compiled them into an electronic inventory.
A comprehensive meta-analysis was performed to identify potential knowledge gaps and redundancies. Findings show that although much 3Rs knowledge exists, its sharing can be improved through better coordination, communication and outreach, and by more emphasis on targeted education and training initiatives. Source: *https://ec.europa.eu/jrc/en/news/reducing-animal-testing-through-better-knowledge-sharing*

NCad advisory report "Transition to non-animal research"

At December 15, 2016, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) has presented its advisory report "Transition to non-animal research – *About the possibilities for phasing out animal procedures and stimulating innovation without laboratory animals*" to the Dutch Minister of Agriculture. The advisory report was written at the Ministers' request. The committee was asked to put together a roadmap for the reduction in the number of procedures involving animals, and identify the possibilities for becoming the world leader in innovation without the aid of laboratory animals in 2025. In the report, NCad identifies clear transition objectives, including the complete phasing out of animal procedures in the field of regulatory safety research by 2025. In response to the advisory report, the Dutch Minister of Agriculture has commissioned the RIVM to draw up an Agenda Innovation without Laboratory Animals for regulatory safety testing , in collaboration with other relevant institutes.



Sources: *https://www.ncadierproevenbeleid.nl/documenten/rapport/2016/12/15/ncad-opinion-transition-to-non-animal-research*, and *https://www.rijksoverheid.nl/.../12/.../kamerbrief.../kamerbrief16188723+(4).pdf* (in Dutch).



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