Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport

Subjects:

Chemical substances

- Proposal to introduce detailed classification criteria based on in vitro and in silico methods into the GHS
- OECD Guidance Document on Good In Vitro Practices (GIVIMP)
- Publication: toxicogenomics as a useful, non-animal tool for risk assessment of chemicals
- Publication: Head skeleton malformations in zebrafish (Danio rerio) embryos to assess adverse effects of mixtures of compounds

Other news and developments

- EUROoC network for joint European organ-on-a-chip research

RIVM 3R's *Quarterly*

November 2018

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

Proposal to introduce detailed classification criteria based on *in vitro* and in silico methods into the GHS

GHS (Globally harmonized system of classification and labelling of chemicals) contains criteria for classification and labelling of substances and mixtures for several hazard classes. The GHS system is included in the chemical legislation of many countries including the CLP legislation in Europe. The criteria are predominantly based on animal tests and human data whereas *in vitro* or *in silico* methods are hardly used. Therefore, a Working Group led by the United Kingdom and the Netherlands was established to fully incorporate non-animal test methods.

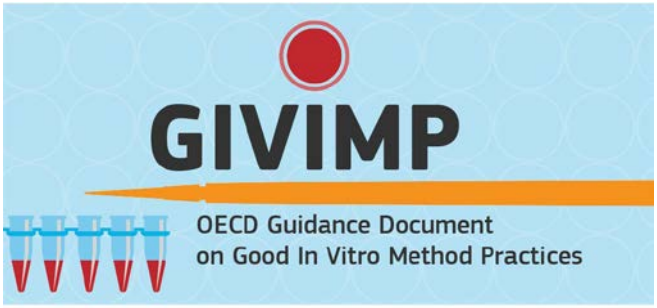
As a first step, the criteria on skin irritation and corrosivity were discussed. An update was developed and recently submitted to the ECOSOC Sub-Committee of Experts on the GHS for adoption in December 2018.

The submission contained a working document with an overview of the proposal and the required changes of the GHS criteria. Further, an informal paper was submitted containing the revised text. This new text provides criteria and guidance on classification based on *in vitro* and *in silico* methods including a conclusion for no classification, giving higher weight to *in vitro* methods. Also guidance was developed on the use of human data and other existing skin data in animals.

The working document and informal paper can be found at: *http://www.unece.org/fileadmin/DAM/trans/doc/2018/dgac10c4/ST-SG-AC.10-C.4-2018-29e.pdf* and *http://www.unece.org/fileadmin/DAM/trans/doc/2018/dgac10c4/UN-SCEGHS-36-INF6e.pdf*

OECD Guidance Document on Good In Vitro Practices (GIVIMP)

In September 2018, OECD adopted the Guidance Document on Good In Vitro Method Practices (GIVIMP) guiding the development and implementation of *in vitro* methods for regulatory use in human safety assessment. The aim of the guidance document is to reduce the uncertainties in predictions derived from cell and tissue-based *in vitro* methods by applying all necessary good scientific, technical and quality practices and by covering the whole range from *in vitro* method development to *in vitro* method implementation for regulatory use. Adherence to this guidance by developers is expected, among others, to facilitate final validation of newly developed *in vitro* methods, but it also applies to *in vitro* methods already accepted by the OECD. RIVM contributed to Chapter 6 of the GIVIMP guidance document, while also checking the whole of the document content. The GIVIMP guidance document can be found at: *http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2018)19&doclanguage=en.*



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Chemical substances

Publication: toxicogenomics as a useful, non-animal tool for risk assessment of chemicals

RIVM published an *in vitro* toxicogenomics tool that is useful for detecting modes of action in chemical risk assessment. The article was published in Food and Chemical Toxicology. Insight into the mode of action (MOA) of a chemical in humans leads to better understanding of its putative adverse health effects. In addition, MOA information has potential to greatly enhance the use of non-animal methods for risk assessment, as it will ultimately enable extrapolation from initiating events to adverse effects. RIVM previously published on this *in vitro* toxicogenomics 'comparison approach', using it to categorize (non-) genotoxic carcinogens according to similarities in their proposed modes of action. The underlying concept is universally applicable to environmental and pharmaceutical chemicals.

In the current publication, RIVM made the comparison approach generally applicable allowing comparison of outcomes across different studies. Using the Open TG-GATEs database, we were able to improve several aspects of the comparison approach while leaving its concept unchanged. The study demonstrates the applicability and the usefulness of the comparison approach as a tool in mechanism-based risk assessment. The publication can be accessed through *https://doi.org/10.1016/j.fct.2018.08.007*. The previous publications can be found at: *https://doi.org/10.1007/s00204-014-1368-6* and *https://doi.org/10.1007/s00204-012-0883-6.*

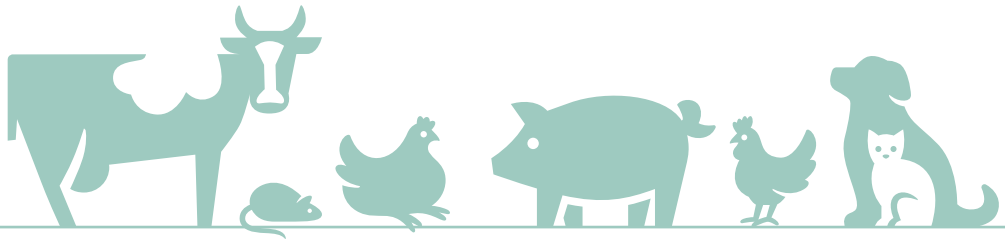
Publication: Head skeleton malformations in zebrafish (Danio rerio) embryos to assess adverse effects of mixtures of compounds

RIVM published in Archives in Toxicology results of the EU-EuroMix project describing a method to determine head skeleton malformations in zebrafish embryos, which could be applied to assess the effects of mixtures. Zebrafish were exposed to test compounds 0–120 hpf and alcian blue cartilage staining at 120 hpf, focusing on the head skeleton. Zebrafish embryos until this age have an early stage of development of the nervous system, which does not enable them to perceive pain or distress. In present legislation, they are therefore not considered as organisms. As such, zebrafish embryos do not require a license for experimental studies, and can therefore be used as an alternative whole organism enabling analysis of complex biological processes. Reference compounds cyproconazole, flusilazole, metam, and thiram induced distinctive phenotypes in the head skeleton between the triazoles and dithiocarbamates. Of many evaluated parameters, the Meckel's–palatoquadrate (M–PQ) angle was selected for further assessment, based on the best combination of a small confidence interval, an intermediate maximal effect size and a gentle slope of the dose–response curve with cyproconazole and metam. An additional set of test compounds associated with the induction of craniofacial malformations or cleft palate were tested for M–PQ effects. This additional set included hexaconazole, all-trans-retinoic acid, AM580, CD3254, maneb, pyrimethanil, imidacloprid, pirimiphos-methyl, 2,4-dinitrophenol, 5-fluorouracil, 17alpha-ethynylestradiol (EE2), ethanol, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), PCB 126, methylmercury, boric acid, and MEHP. Most of the results obtained with these compounds showed a dose–response for M–PQ effects. Application of the assay in mixture testing was provided by combined exposure to cyproconazole and TCDD through the isobole method, supporting that in this case the combined effect can be modelled through concentration addition. The full article can be downloaded at: *https://doi.org/10.1007/s00204-018-2320-y*

Other news and developments

EUROoC network for joint European organ-on-a-chip research

RIVM participates as a partner organization in a European research network to promote organ-on-a-chip technology. The network is coordinated by the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB. Organ-on-a-chip systems enable the recapitulation of human organ tissues on a very small scale. They are regarded as a future alternative to animal models and as a technology with great potential for pharmaceutical research and personalized medicine. Since the development of organ-on-a-chip systems requires skills and expertise from various disciplines, the primary aim of the EUROoC network is the interdisciplinary training of young scientists. The full press release of the EUROoC network can be found at: *https://www.igb.fraunhofer.de/content/dam/igb/en/documents/press-releases/2018/1809_PR_EUROoC_en.pdf*



Published by

National Institute for Public Health and the Environment, RIVM
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

November 2018

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010964