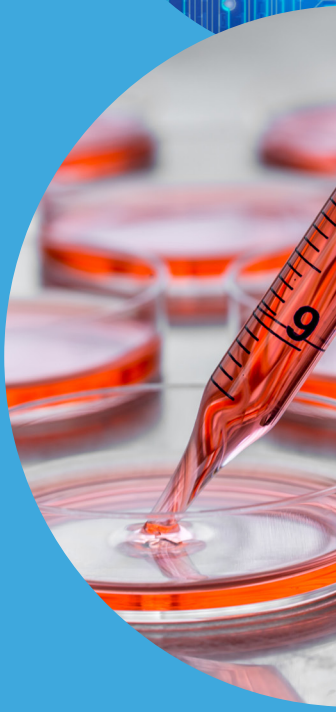
# BOOK OF ABSTRACTS

**RegToxInVitro**

**Dissemination and implementation of the OECD *in vitro* and *in silico* methods applicable to the safety and risk assessment of the chemicals, food, and feed**



**May 24 - 25, 2021**

# Book of Abstracts

## International scientific symposium

## RegToxInVitro – Dissemination and implementation of the OECD *in vitro* and *in silico* methods applicable to the safety and risk assessment of the chemicals, food, and feed

May 24 – 25, 2021 | Bratislava, Slovakia

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## Organized by

efsa European Food Safety Authority FOCAL POINT

Slovenská toxikologická spoločnosť SETOX Slovak Toxicology Society

## Supported by

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MINISTRY OF FOREIGN AND EUROPEAN AFFAIRS OF THE SLOVAK REPUBLIC

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT OF THE SLOVAK REPUBLIC

INSTITUTE OF EXPERIMENTAL PHARMACOLOGY & TOXICOLOGY SLOVAK ACADEMY OF SCIENCES

sR3

MatTek IN VITRO LIFE SCIENCE LABORATORIES

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## About the RegToxInVitro

The goal of this international scientific symposium was to discuss the implementation of innovative and internationally recognized approaches (*in vitro, in silico*) to the toxicity assessment of substances that do not require animal testing and to increase the expert levels of science and research in the field of practical implementation of these methods listed in the official guidelines approved by the OECD. Target scientific communities were from the Visegrad Group countries (Slovak Republic, Czech Republic, Hungary, and Poland) and neighbouring countries of Slovakia (Austria and Ukraine).

The event brought together respected renowned speakers from the Organization for Economic Co-operation and Development (OECD), the European Chemicals Agency (ECHA), the European Food Safety Authority (EFSA), the Slovak Academy of Sciences (SAS), The National Institute of Public Health in Prague (NIPH), Austrian Institute of Technology GmbH (AIT), The National University of Food Technologies (NUFT) in Ukraine and Semmelweis University in Hungary.

The symposium consisted of 3 main parts: theoretical presentations, practical demonstrations (*in vitro* and *in silico*) and a Round table discussion on the implementation of the principles of 3Rs in Central and Eastern Europe.

## Organizers

The event was organized by the Department of Food Safety and Nutrition of the Ministry of Agriculture and Rural Development of the Slovak Republic (MPRV SR) and the Slovak Toxicology Society SETOX. The scientific guarantor of the project was the Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine SAS.

### Department of Food Safety and Nutrition

of the MPRV SR is the Slovak Focal Point for the Scientific and Technical Matters of the EFSA, the National contact point for the PARERE Network and ensures the performance of another 7 contact points with European and international scope. It also fulfills legislation-based responsibilities in the fields of official controls and placing the GMO food and feed on the market, and also organises national and international events focused on food safety in the agri-food chain.

### Slovak Toxicology Society SETOX

is a civic association of toxicologists, affiliated with the Slovak Academy of Sciences. SETOX organises national and international events in the field of toxicology, leads the Slovak National Platform (SNP 3Rs) for the promotion of alternative methods and principles of 3R, and participates in the development of education of Slovak toxicologists, as well as in increasing their qualifications.

## Supporters

The symposium has been financially supported by:

- the Ministry of Foreign and European Affairs of the Slovak Republic (MZVaEZ SR), via the "Mechanism of MZVaEZ SR for joint projects of the Slovak Republic and the OECD"
- the European Food Safety Authority (EFSA), via the financial resources based on the grant agreement with the Slovak Focal Point for the Scientific and Technical Matters of the EFSA.

**The Organisation for Economic Co-operation and Development (OECD)**

is an intergovernmental economic organization whose members recognize the values of democracy and the principles of a market economy. The organization provides a unique forum for finding answers to current challenges, a platform for solving common problems, exchanging experiences and discussion. It currently unites 37 member countries, including the Slovak Republic.

**The European Food Safety Authority (EFSA)**

is a European agency funded by the European Union that operates independently of the European legislative and executive institutions (Commission, Council, Parliament) and EU Member States. It was set up in 2002 following a series of food crises in the late 1990s to be a source of scientific advice and communication on risks associated with the food chain. The agency was legally established by the EU under the General Food Law – Regulation 178/2002.

# Programme

## Monday - May 24, 2021

12:00 – 17:00
**POSTER VIEWING**

12:50 – 13:00
**Official opening of the RegToxInVitro**

13:00 – 16:00
**THEORETICAL LECTURES**

13:00 – 14:30
**Session 1**

**OECD TEST GUIDELINES ON GENOTOXICITY: REGULATORY ACCEPTANCE AND RECENT DEVELOPMENT**
**Dr Nathalie Delrue**
*Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD)*

**TOWARD A BETTER TESTING IN DEVELOPMENTAL NEUROTOXICITY: OECD EFFORTS AND REGULATORY CONSIDERATIONS**
**Dr Magda Sachana**
*Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD)*

**THE OECD QSAR TOOLBOX – A SOFTWARE TO SUPPORT CHEMICAL HAZARD ASSESSMENT**
**Dr Andrea Gissi**
*Computational assessment unit, European Chemicals Agency (ECHA)*

14:30 – 15:00
**Break**

15:00 – 16:00
**Session 2**

**OPENFOODTOX: EFSA'S CHEMICAL HAZARDS DATABASE**
**Dr Jean-Lou Dorne**
*Scientific Committee and Emerging Risks Unit, Department of Risk Assessment and Scientific Assistance, European Food Safety Authority (EFSA)*

**IMPLEMENTATION OF ALTERNATIVE METHODS AND PRINCIPLES OF 3RS INTO THE SCIENCE AND EDUCATION IN SLOVAKIA**
**Dr Helena Kanďárová, ERT**
*Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences (SAS)*

16:00 – 16:10
**Break**

16:10 – 17:00
**ROUND TABLE**

**THE PRINCIPLES OF 3RS IN THE MIDDLE AND EAST EU - WHAT WE HAVE LEARNED AND WHAT WAS ACHIEVED?**
**Dr. Helena Kanďárová, ERT** *(Slovak Republic)*, **Dr Kristina Kejlová** *(Czech Republic)*, **Dr Nataliia Bubalo** *(Ukraine)*, **PD DI Dr Winfried Neuhaus** *(Austria)*, **Dr Györgyi Szabó** *(Hungary)*

## Tuesday - May 25, 2021

8:00 – 12:00
**POSTER VIEWING**

9:00 – 12:00
**PRACTICAL DEMONSTRATIONS**

**DEMONSTRATION OF THE SELECTED *IN VITRO* METHODS ADOPTED BY OECD FOR TOPICAL TOXICITY TESTING**
**Dr Silvia Letašiová** and **Dr Jan Markus**
*MatTek In Vitro Life Science Laboratories s.r.o.*

10:15 – 10:30
**Break**

**OECD QSAR TOOLBOX**
**Dr Andrea Gissi**
*Computational assessment unit, European Chemicals Agency (ECHA)*

**EFSA OPENFOODTOX DATABASE**
**Dr Jean-Lou Dorne**
*Scientific Committee and Emerging Risks Unit, Department of Risk Assessment and Scientific Assistance, European Food Safety Authority (EFSA)*

## About the speakers

### Dr Nathalie Delrue

*Policy Analyst*
Environment Health and Safety Division, Environment Directorate, **Organisation for Economic Co-operation and Development** (OECD), Paris, France

Dr Delrue is an Administrator of the Test Guidelines Programme at the OECD. She joined the OECD Secretariat in 2006 and is managing the development of new or updated Test Guidelines related to human health, in particular skin sensitization, genotoxicity, and carcinogenicity. She is in charge of the coordination of the Adverse Outcome Pathway development programme, initiated at OECD in 2012. Before joining the OECD she worked in the Toxicological Expertise Unit of the Chronic Risk Direction at INERIS (French Institute for Industrial Environment and Risks). She was in charge of hazard identification/risk assessment for human health in various international chemical programmes (EU, OECD). She holds a doctorate degree in pharmaceutical sciences from University René Descartes (Paris V) and two Master's degrees, one in Biological and Medical Science (Paris V) and one in Water, Health and Environment (Bordeaux 2).

### Dr Magda Sachana

*Policy Analyst*
Environment Health and Safety Division, Environment Directorate, **Organisation for Economic Co-operation and Development** (OECD), Paris, France

Dr Magda Sachana is an Administrator within the Environment Health and Safety Division of the OECD's Environmental Directorate since 2015. She manages the development and implementation of policies in the field of chemical safety and contributes to the OECD Test Guidelines, Pesticide and Hazard Assessment Programmes. Dr Sachana among other projects is managing the OECD project on the *in vitro* battery for developmental neurotoxicity since 2017.

## Dr Andrea Gissi

*Scientific Officer*
Computational assessment unit, **European Chemicals Agency** (ECHA), Helsinki, Finland

Dr Gissi has been working in the computational assessment unit of the ECHA since 2013. His main activities include the assessment of the reliability of QSAR studies submitted in REACH registration and the project and product management of the OECD QSAR Toolbox. Dr Gissi has a Ph.D. in computational ecotoxicology and a master's degree in medicinal chemistry.

## Dr Jean-Lou Dorne

*Senior Scientific Officer*
Scientific Committee and Emerging Risks Unit, Department of Risk Assessment and Scientific Assistance, **European Food Safety Authority** (EFSA), Parma, Italy

Dr. Dorne has been working since 2006 in EFSA as a Senior Scientific Officer in the Scientific Committee and Emerging Risks unit. Previously, he spent 9 years at the University of Southampton between a PhD and 5 years of postdoctoral research in the UK on "human variability in kinetics and metabolism and refinement of uncertainty factors for chemical risk assessment". His work focuses on chemical risk assessment and toxicology.

Key areas of work include:

1. The development and publication of EFSA's chemical hazards database: OpenFoodTox and TK plate an open source platform integrating kinetic models for humans and animals.
2. Implementation of New Approach Methodologies (NAMs) in risk assessment.
3. Mixture risk assessment for humans, animals and the environment.
4. Training programmes in risk assessment across Europe and in other countries.
5. Over 120 peer reviewed publications, 100 EFSA outputs and 6 book chapters (h-index:34).

## Dr Helena Kandárová, ERT

*Senior Scientist at SAS and Lecturer at STU*
Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, **Slovak Academy of Sciences** (SAS) & Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, **Slovak University of Technology in Bratislava** (STU), Bratislava, Slovakia

Dr Kandárová, ERT is a Senior Scientist at the Institute of Pharmacology and Toxicology, Centre of Experimental Medicine, Bratislava. She also holds a position at the Institute of Biochemistry and Microbiology at the Faculty of Chemical and Food Technology at the Slovak University of Technology in Bratislava. From 2007 - 2018, Dr Kandárová, ERT held the position of Senior Scientist and General Acting Manager for EU at MatTek Corporation, USA. She established MatTek In Vitro Life Science Laboratories in Europe and led the company in the position of Executive Director between 2009 - 2018. Dr Kandárová, ERT is an executive board member in several European toxicology-oriented organizations and a member of the international expert panels. In 2020 she has been elected as president of the European Society for Toxicology in Vitro (ESTIV). Dr Kandárová, ERT has been involved in many international projects aiming at the validation of 3D reconstructed human tissue models for topical toxicity and phototoxicity testing of chemicals, cosmetics, pesticides, and medical devices. Dr Kandárová, ERT is the co-author of several internationally implemented protocols that were adopted also into the OECD and ISO 10993 guidelines. She published over 55 papers and book chapters and obtained several scientific awards related to the research aiming into the development and validation of alternative methods.

## Dr Kristina Kejlová

*Head of Unit*
Unit for Alternative Toxicological Methods, Center of Toxicology and Health Safety, **The National Institute of Public Health in Prague** (NIPH), NRL for Experimental Immunotoxicology, Prague, Czech Republic

Dr Kejlova is focused on the validation and implementation of *in vitro* methods for skin/eye irritation, phototoxicity, genotoxicity, endocrine

disruption, and sensitization. She is a member of numerous OECD Expert Groups, of the National Committee for the Protection of Animals Used for Scientific Purposes at the Ministry of Agriculture, of the Committee for Animal Welfare at the Ministry of Health, nominated as a single point of contact to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation (PARERE Network) and a former member of the ECVAM Scientific Advisory Committee.

**Dr Nataliia Bubalo**
*Researcher and Senior Teacher*
Department of Fats, Perfumery and Cosmetic Products Technology, **The National University of Food Technologies** (NUFT) & L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, **Ministry of Health**, Kyiv, Ukraine

Dr Bubalo worked at L. I. Medved's Research Center of Preventive Toxicology Food and Chemical Safety in the field of general toxicology in 2008-2018. She established GLP laboratories of alternative research methods, implemented *in vitro* methods in this Center, and created a Ukrainian 3R Center. Nowadays, she has been working at the National University of Food Technologies as head of course Basics of Toxicology. She actively works with the Ukrainian Government to implement new cosmetics legislation which bans animal tests.

**Dr Györgyi Szabó**
*Associate Professor and President of the Animal Welfare Body*
Animal Welfare Body and Department of Surgical Research and Techniques, **Semmelweis University**, Budapest, Hungary

Dr Szabó is a biologist who has been working for Semmelweis University since 2011. She was elected in 2013 to be the secretary of Semmelweis University's Animal Welfare Body and she is the president of the Body since October 2020. She organized 12 "Experimental animals -

animal experiments" courses for educating the researchers, assistants, and technicians to become competent in this field, and more than 800 people participated in these courses. Dr Szabó is also the board member of EUSAAT (European Society for Alternatives to Animal Testing) and one of the founding members of EALAS (European Academy of Laboratory Animal Surgery).

**PD DI Dr Winfried Neuhaus**
*Principal Scientist at AIT and President of EUSAAT*
Competence Unit Molecular Diagnostics, **Austrian Institute of Technology GmbH** (AIT), Vienna, Austria &
**European Society for Alternatives to Animal Testing** (EUSAAT), Luftenberg an der Donau, Austria

PD DI Dr Neuhaus studied Food and Biotechnology at the University of Natural Resources and Life Sciences. After the establishment of an industrial plant for enzymatic lactose hydrolysis based on hollow-fiber reactors, he started his Ph.D. in Pharmacy at the Department of Pharmaceutical Chemistry of the University of Vienna. After his time as University Assistant and lecturer at the FH Campus Biotechnology, he headed the group "Alzheimer and Blood-Brain Barrier Research" in the pharmaceutical company PharmaCon for 2 years. Before he started at AIT in 2016, he worked at the University Hospital Würzburg for 6 years and was at the same time PI at the University of Vienna and scientist at the Medical University of Vienna. During his time in Würzburg, he was granted his habilitation in Molecular Medicine. His main research field is biological barriers in health and disease with a current focus on the blood-brain and the blood-saliva barrier. Currently, he is teaching at five Universities and is the president of EUSAAT and coordinator of EU3Rnet.

## Dr Silvia Letašiová

*Managing Director and Senior Scientist*
**MatTek In Vitro Life Science Laboratories s.r.o.**, Bratislava, Slovakia

Dr Letasiova is the managing director and senior scientist at MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia. She has a background in biochemistry and microbiology and holds a doctoral degree in biochemistry. She joined MatTek in 2009 as a production manager for EpiDerm and is also responsible for R&D projects in the area of skin/eye irritation, corrosion, and sensitization.

## Dr Jan Markus

*Senior Scientist and Production Manager*
**MatTek In Vitro Life Science Laboratories s.r.o.**, Bratislava, Slovakia

Dr Markus is the production manager and senior scientist at MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia. He has a background in molecular biology and virology and holds a doctoral degree in experimental oncology. He is responsible for the production and further development of reconstructed tissue models of the small intestine and respiratory pathways. He is also actively involved in the development and validation of new tests aimed at the reduction of *in vivo* testing. His areas of expertise include cell and tissue cultures, molecular biology, transcriptional gene regulation, and oncology.

# Abstracts

**International scientific symposium**

**RegToxInVitro – Dissemination and implementation of the OECD *in vitro* and *in silico* methods applicable to the safety and risk assessment of the chemicals, food, and feed**

*Note: The authors are solely responsible for the scientific content of the abstracts.*

## OECD TEST GUIDELINES ON GENOTOXICITY: REGULATORY ACCEPTANCE AND RECENT DEVELOPMENT

**DELRUE N.**

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The OECD is committed to harmonising international testing methods and Good Laboratory Practices used for chemical safety assessment to ensure high quality and reliable data while also reducing duplicative testing and the use of animals in such tests and facilitating the sharing of test data across governments. The presentation described the instruments of the OECD Mutual Acceptance of Data system and the Test Guideline (TG) development process in particular, with a focus on genotoxicity Test Guidelines.

To adequately cover all the genetic endpoints, the use of test batteries is mandatory in most jurisdictions because no individual test can provide information on all genetic endpoints. In 5 years, the set of OECD Genetic Toxicology Test Guidelines has been significantly updated, with TGs being developed or updated, and others deleted. This update of the set of genotoxicity TGs has been based on progress in science and increased knowledge of the mechanisms leading to genetic toxicity, increased experience with the use of the tests, and economic and animal welfare considerations. The presentation also described related projects currently under development at OECD and new genotoxicity tests which may become available in the coming years.

Finally, an overview of existing Adverse Outcome Pathways related to genotoxicity was presented to illustrate the development of other approaches available to aid risk assessors in their work to use all existing information on the effects of chemicals.

## TOWARD A BETTER TESTING IN DEVELOPMENTAL NEUROTOXICITY: OECD EFFORTS AND REGULATORY CONSIDERATIONS

**SACHANA M.**

*Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD), Paris, France*
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Characterization of the potential chemical-induced developmental neurotoxicity (DNT) hazard is considered for risk assessment purposes by

many regulatory sectors. However, due to test complexity, difficulty in interpreting results and the need for substantial resources, the use of the *in vivo* DNT test guidelines has been limited and animal data on DNT is scarce. To address challenging endpoints such as DNT, the OECD chemical safety programme, has been working lately towards the development of integrated approaches for testing and assessment (IATA) that rely on a combination of multiple results from different methods (e.g. *in vitro*, *in silico*, and non-mammalian *in vivo* models) that are supported by mechanistic knowledge organized according to the adverse outcome pathway (AOP) framework.
In 2017, the OECD convened a dedicated OECD expert group to develop a guidance document on the application and interpretation of data derived from a DNT testing battery that relies on key developmental processes and is complemented by zebrafish assays.
The presentation highlighted the main milestones achieved since the launch of the OECD DNT project and explores the contribution of experimental data and case studies to the development of the guidance document. Finally, the audience was informed about the efforts to increase regulatory uptake of the DNT *in vitro* battery of assays.

## THE OECD QSAR TOOLBOX – A SOFTWARE TO SUPPORT CHEMICAL HAZARD ASSESSMENT

**GISSI A.**

*Computational assessment unit, European Chemicals Agency (ECHA), Helsinki, Finland*

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The Toolbox is a free software application that supports reproducible and transparent chemical hazard assessment. As a freely available computational tool, it promotes the use of non-test methods and minimizes unnecessary animal testing without reducing the safety of human health and the environment. It is intended to be used by governments, the chemical industry, and other stakeholders.
Using the Toolbox, the users can:

- **Find experimental data** - With over 2 500 000 data points for almost 100 000 structures from around 60 databases, the Toolbox is an invaluable tool to find publicly available experimental data including details on the experimental test.
- **Find analogues, build and assess categories** - the Toolbox can be used to find data-rich analogues based on the knowledge for

structural characteristics (alerts from profilers) and chemical properties. The Toolbox can be also used to assess the consistency of a category, the starting point for read-across justification.

- **Account for metabolism** - The assessment of metabolism and transformation products is a crucial step in chemical risk assessment. The Toolbox can be used to explore experimental metabolic maps or to simulate the metabolism and degradation of chemical substances in different organisms and conditions.
- **Fill data gaps from analogues** – the toxicity of a substance can be predicted by using data from analogues. Classical read-across or trend analysis can be used for this purpose. Furthermore, the Toolbox also includes external QSAR models.

During the practical session were shown functionalities mentioned in the presentation using the Toolbox and the participants could try to run the Toolbox on their computers.

**OECD QSAR Toolbox - additional resources:**

- QSAR Toolbox for download
- Manuals
- Additional information on QSAR Toolbox

## OPENFOODTOX: EFSA'S CHEMICAL HAZARDS DATABASE

**DORNE J-L.**

*Scientific Committee and Emerging Risks Unit, Department of Risk Assessment and Scientific Assistance, European Food Safety Authority (EFSA), Parma, Italy*

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Since its creation in 2002, the EFSA has produced over 2000 Scientific Opinions as risk assessments, Statements, and Conclusions for over 5000 substances through the work of its Scientific Panels, Units and Scientific Committee. Over the last 3 years, EFSA has been publishing OpenFoodTox, an open-source toxicological database for all substances evaluated by EFSA which is available for download and data visualization. OpenFoodTox provides substance characterisation, links to EFSA's outputs, applicable legislations regulations, and a summary of hazard data for human health, animal health, and ecological assessments. The data model uses OECD harmonized templates (OHTs) which allows to structure the data and facilitates data sharing with all relevant stakeholders including the

risk assessment community, such as sister agencies, international scientific advisory bodies, industry, and others.
This short course provided a description of OpenFoodTox and its functionalities as well as examples of applications of OpenFoodTox in chemical risk assessment including new quantitative structure-activity relationship (QSAR) models and their future developments.
The second part of the course provided the user with training to users particularly to search the database and search its content for specific substances.

**EFSA OpenFoodTox Database - additional resources:**

- OpenFoodTox: EFSA's chemical hazards database for download
- About OpenFoodTox
- Infographic
- Editorial: OpenFoodTox: EFSA's open source toxicological database on chemical hazards in food and feed
- Recent Review on OpenFoodTox: EFSA's OpenFoodTox: An open source toxicological database on chemicals in food and feed and its future developments. Environmental International

## IMPLEMENTATION OF ALTERNATIVE METHODS AND PRINCIPLES OF 3RS INTO THE SCIENCE AND EDUCATION IN SLOVAKIA

**KANĎÁROVÁ H.[1,2], MILEC L.[3]**

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*[2]Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava (STU), Bratislava, Slovakia*
*[3]Slovak Focal Point for the Scientific and Technical Matters of the EFSA, Ministry of Agriculture and Rural Development of the Slovak Republic, Bratislava, Slovakia*
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The need for implementation of the Directive 2010/63/EU on the protection of animals used for scientific purposes led in Slovakia to many discussions on how to cope with the aspects of this complex EU law effectively. The National Contact Point for implementing the Directive 2010/63 is the State Veterinary and Food Administration of the Slovak Republic (ŠPVS SR) affiliated to the Ministry of Agriculture and Rural Development of the Slovak Republic (MPRV SR). It collaborates with the National Committee on the protection of animals used for scientific purposes established by the MPRV SR. Department of Food Safety and Nutrition is the National

contact point for the Preliminary Assessment of REgulatory Relevance (PARERE Network) activities (Article 47 of the Directive 2010/63/EU). Several external experts support the MPRV SR and help with the processes of the implementation of this very complex regulation.
The idea to establish a national 3Rs centre that could conduct practical work and be an information resource on alternatives was under discussion among the Slovak scientists, regulators, and industry since 2015. The concept of a centre was presented already in 2016 and 2017 at toxicology meetings, but it took another 2 years to launch the national platform under the name The Slovak National Platform for 3Rs (SNP 3Rs). The current function of SNP 3Rs is to develop and encourages collaboration between individuals and organisations from various fields to implement 3Rs and later on to grow into a 3R centre. Activities of SNP 3Rs are at the national level supported by the MPRV SR, Slovak Toxicology Society SETOX, individual members from academia and governmental organisations involved in the implementation of the EU legislation on animal welfare. At the international level, SNP 3Rs collaborates closely with the Czech 3Rs centre located at the The National Institute of Public Health (NIPH) in Prague. In 2020, SNP 3Rs joined the European 3Rs centres' network affiliated to the organisation European Society For Alternatives To Animal Testing (EUSAAT).
The platform representatives are actively involved in the education as well as in development, validation and implementation of alternative methods into the regulatory frameworks. Many of them are members of national and international committees and associations relevant to the 3Rs. One of the long-term goals of the platform is to secure funding from national sources to support the activities and work of experts that is necessary for the successful implementation of requirements laid down in the regulation 2010/63/EU.

## DEMONSTRATION OF THE SELECTED *IN VITRO* METHODS ADOPTED BY OECD FOR TOPICAL TOXICITY TESTING

**LETAŠIOVÁ S., MARKUS J.**
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The presentation provided a brief overview of methods utilizing 3D in vitro reconstructed human tissues. The initial part provided a background on the particular tissue models.
The practical presentation demonstrated:

1. step-by-step procedures that are necessary for successful setup of experiments with *in vitro* intestinal model EpiIntestinal
2. detailed demonstration of Eye Irritation testing protocol using EpiOcular model (OECD 472)

## THE PRINCIPLES OF 3RS IN THE MIDDLE AND EAST EU – WHAT WE HAVE LEARNED AND WHAT WAS ACHIEVED?

Round table debaters: **BUBALO N.**[1,2] *(Ukraine)*, **KEJLOVÁ K.**[3] *(Czech Republic)*, **SZABÓ G.**[4] *(Hungary)*, **NEUHAUS W.**[5,6] *(Austria)*

*Moderator:* **KANĎÁROVÁ H.**[7,8] *(Slovak Republic)*

[1]*Department of Fats, Perfumery and Cosmetic Products Technology, The National University of Food Technologies (NUFT), Kyiv, Ukraine;*
[2]*L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Kyiv, Ukraine;*
[3]*Unit for Alternative Toxicological Methods, Center of Toxicology and Health Safety, The National Institute of Public Health in Prague (NIPH), NRL for Experimental Immunotoxicology, Prague, Czech Republic;*
[4]*Animal Welfare Body and Department of Surgical Research and Techniques, Semmelweis University, Budapest, Hungary;*
[5]*Competence Unit Molecular Diagnostics, Austrian Institute of Technology GmbH (AIT), Vienna, Austria;*
[6]*European Society for Alternatives to Animal Testing (EUSAAT), Luftenberg an der Donau, Austria;*
[7]*Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences (SAS), Bratislava, Slovakia;*
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Every year, more than 100 million animals are used worldwide for scientific research purposes and regulatory toxicity testing. These tests include both safety and efficacy testing of substances like cosmetic products, industrial chemicals, drugs, pesticides, food additives, biocides, medical devices. Animals are also used to train or test surgical techniques, environmental changes; or in other ways of altering the live animal's physiology and/or behaviour.
While the safety testing for regulatory purposes is highly regulated and the use of animals strictly controlled, efficacy and "proof of concept" testing occurring in the universities and research centres appears to be less regulated.

Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for the scientific purpose in the Article 47 states that:

1. The Commission and the Member States shall contribute to the development and validation of alternative approaches which could provide the same or higher levels of information as those obtained in procedures using animals, but which do not involve the use of animals or use fewer animals or which entail less painful procedures, and they shall take such other steps as they consider appropriate to encourage research in this field.
2. Member States shall assist the Commission in identifying and nominating suitable specialised and qualified laboratories to carry out such validation studies.
3. After consulting the Member States, the Commission shall set the priorities for those validation studies and allocate the tasks between the laboratories for carrying out those studies.
4. Member States shall, at the national level, ensure the promotion of alternative approaches and the dissemination of information thereon.
5. Member States shall nominate a single point of contact to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation.
6. The Commission shall take appropriate action with a view to obtaining international acceptance of alternative approaches validated in the EU.

The Round table discussed which concrete steps have been undertaken in Austria, the Czech Republic, Hungary, Slovakia, and Ukraine to address these requirements. It will discuss national 3Rs initiatives, a collaboration between the scientists and regulators, funding of research, education, and dissemination of 3Rs, and involvement of the central and east EU countries in the validation process of New Approach Methodologies (NAMs) in toxicology.

## THE UKRAINIAN 3RS CENTER. MISSION, GOAL, RESULTS

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At the beginning of 2020, on the initiative group of scientists who work in vitro and *in silico* toxicological fields Ukrainian 3Rs Center (UN3RC) was created. We invited a group of scientists who work *in vivo* toxicological field (DNT, DART, subchronic, chronic toxicity) to be members of the UN-3RC.

The result of a collaboration between these two groups was implementing three in vitro methods (OECD 431, OECD 439, OECD 492) on a facilities L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health. These activities will help save the lives of 120 animals annually.

We strongly believe that only collaboration that mode gives the effective way to use alternative methods like in vitro and *in silico*, which relate to the first postulate of 3Rs principles. But, in some study where animals strongly involved and methods mentioned above are far from the full implementation (such as DNT, DART, Chronic Toxicity Tests etc.) should be used the other two postulates (Reduction and Refinement) and methods which support them. In this case, people who closely work in these areas (*in vivo*) and knows alternative methods can realise it more effective.

The application of in vitro method is one step to GHS implementation in Ukraine. Seeing, The Ukrainian hazard classification system (UAC) is in place and it is solely based on results of *in vivo* studies. The main challenge encountered was the lack of a regulatory basis for alternative methods application and interpretation of the results for classification in accordance with UAC.

Implementing the in vitro tests made it possible to adopt a new Regulation of Cosmetics product in Ukraine. According to this legislation, testing cosmetics on animals is forbidden.

3R principles promote through education. Module "The alternative methods. 3R principles" was included in Toxicological Course at The National University of Food Technologies. Their participants are future technologist of cosmetics products. Also, it is a part of a Toxicology Course for specialist (people from the government, industry, regulators, scientists).

The Ukrainian 3Rs Center is a platform for popularisation and implementing alternative methods and promotes 3Rs principles.

## EFFECT OF THE CYANOGENIC GLYCOSIDE AMYGDALIN ON THE INFLAMMATORY PATHWAY

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The immune response of a vascularized organism to any disease process involves inflammation. As part of effective defense, but also protection against self-harm, the regulation by inflammatory mediators accompanies this relatively complex cascade. However, as these cause adverse painful inflammatory symptoms, the metabolic pathways have become the target of several drugs. In an effort to alleviate one problem, new ones have emerged - loss of maintenance of the physiological state of the gastric mucosa, loss of protection of the myocardium and others. Scientific research has thus turned its attention to the search for alternatives in the form of natural substances, including amygdalin. This cyanogenic glycoside is not rare in nature, we can find it e.g. in almonds with bitter phenotype or in apricot pits. However, its applicability is limited by toxicity at different doses, by the route of administration due to the diverse intestinal microbiome of the individual, and, last but not least, by the lack of knowledge of the exact anti-inflammatory mechanism of amygdalin action. The subject of our work was therefore an aspect of the biochemical structure of this substance, toxicity and its treatment, and the activity of amygdalin in inflammatory pathways. Finally, several studies have provided evidence of the anti-inflammatory effect of amygdalin through inhibition of transcriptional signals leading to the initiation of pro-inflammatory cytokine production, in particular IL-1α, IL-1β, IL-6, IL-9, TNF-α and MCP-1. This cyanogenic glycoside within the inflammatory pathway of arachidonic acid has been shown to inhibit the expression of COX-2 mRNA and the metabolite prostaglandin $E_2$, and also prevented the formation of inducible NO synthase involved in inflammation. In addition to these effects, amygdalin may suppress the development of atherosclerosis and participate in the control of inflammatory markers by suppressing the phospho-MAPK/ phospho-AP-1/ NF-κB p65 signaling pathway and thus also plays a strong anti-inflammatory role in atherosclerosis. However, the topic is still not sufficiently studied, so further research is needed.

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## THE IMPACT OF TRIBULUS TERRESTRIS EXTRACT ON MICE LEYDIG CELLS *IN VITRO*

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Nowadays, we recognize plenty of exogenous factors, which may interact with human and wildlife reproductive health, including heavy metals, endocrine disruptors, and other xenobiotics. The majority of their negative effects, such as decreased testis weights, prostate cancer, poor semen quality, and insufficient production of steroid hormones, are frequently linked to damage of essential cellular organelles or disruptions to the processes responsible for normal reproductive functions. An alternative therapy mediated by medicinal herbs may be one of the effective ways to protect the reproductive system. The most beneficial effect of medicinal herbs is related to the content of biologically active substances that can improve spermatogenesis, steroidogenesis, increase sperm count and motility, and in some cases, reverse the overall subfertility. One of the most popular herbs to treat subfertility, venereal diseases, and sexual weakness is *Tribulus terrestris*. *T. terrestris* is an annual herb of the *Zygophyllaceae* family with rich content of phytoconstituents such as flavonoids, alkaloids, saponins, lignin, amides, or glycosides. However, the unique strength is furostanol bisglycosides - protodioscin and protogracillin. The aim of the present *in vitro* study was to evaluate the potential effect of *T. terrestris* on the cell viability and steroidogenesis in mice Leydig cells. TM3 cells were cultured in the presence of different doses of *T. terrestris* (37.5; 75; 150 and 300 µg/mL) during 24 h exposure. Quantification of the cell viability was evaluated using the metabolic activity (alamarBlue) assay, while the level of steroid hormone secretion was determined by enzyme-linked immunosorbent (ELISA) assay. According to our results, at 150 µg/mL of *T. terrestris* a slight increase in Leydig cells viability was observed. Overleaf, at the highest dose (300 µg/mL) of experimental extract, a moderate decrease was recorded. However, administered doses did not affect cells viability

significantly. Exposure to applied doses (37.5-150 µg/mL) of *T. terrestris* enhanced progesterone production, but not significantly. Measurements of testosterone production have confirmed significant ($P<0.05$) growth at 150 µg/mL followed by non-significant reduction at 300 µg/mL. Considerably more detailed and systematic research is required for a better understanding of reproductive benefits associated with *Tribulus terrestris* action.

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## 3RS CENTRE CZECH REPUBLIC AT NATIONAL INSTITUTE OF PUBLIC HEALTH IN PRAGUE

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Czech Republic has implemented the Directive 2010/63/EU on the protection of animals used for scientific purposes into the Act No. 359/2012, amending Act No. 246/1992, on the protection of animals against cruelty. The new directive and amended act include the protection of animals used for scientific purposes and for toxicological testing. The aim of the new directive is the reduction of animals in tests (Reduction), refinement limiting their suffering (Refinement) and replacement of animal testing by alternative *in vitro* methods utilizing cells and tissues of human origin (Replacement). In 2013, the Ministry of Agriculture in the Czech Republic has nominated the National Institute of Public Health (NIPH) in Prague as the contact point, and the National Reference Laboratory for Experimental Immunotoxicology at the Centre of Toxicology and Health Safety, headed by Dagmar Jirova, MD, Ph.D., as a specialized National reference laboratory (NRL). Following a selection procedure, NRL has been appointed into the network of selected national reference laboratories (EU NETVAL), participating on validation of alternative *in vitro* methods under a supervision of the EU Reference Laboratory for alternatives to animal testing EURL-ECVAM, an integral part of the Joint Research Centre (JRC),

the science and knowledge service of the European Commission located in Ispra, Italy. The presented poster summarizes the range of activities of the contact point and the National reference laboratory.

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## DETERMINATION OF THE EFFECT OF BISPHENOLS ON THE H295R CELLS VIABILITY AND TESTOSTERONE PRODUCTION *IN VITRO*

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Heavy metals, endocrine disruptors, and other xenobiotics are only a few examples of exogenous factors that may interfere with human and wildlife reproductive health. Bisphenol A (BPA) is currently produced in large amounts. According to several reports, it is one of a growing list of so-called endocrine disrupting chemicals. Following the decision of a number of regulatory agencies to ban BPA from food packaging, a number of bisphenol analogs (BPA-A) have been developed to replace BPA in a variety of applications. Bisphenol S (BPS) and bisphenol F (BPF) have replaced BPA in thermal receipt paper and epoxy resin materials. However, little is known about the possible negative effects of these widely used bisphenols, though some of them seem to affect hormone systems in the same way as BPA does. The aim of this *in vitro* study was to evaluate the effect of bisphenol F and bisphenol S on cell viability and testosterone production in H295R cells. During a 24-hour incubation period, the H295R cell line was exposed to different doses of selected bisphenols (0,01 - 100 μM). The metabolic activity (alamarBlue) assay was used to determine H295R cell viability, while the enzyme-linked immunosorbent assay (ELISA) was used to determine testosterone production. Our findings indicated that lower experimental doses of BPS have no significant effect on cell viability, but we did observe a significant increase in cell viability in H295R cells treated with 0.1 M μBPF. A significant decrease in cell viability was observed at the highest dose (100 μM) of BPS, and we also observed a significant decrease in cell viability in cells treated with 10-100 μM BPF. Significant (P0.05) growth in

testosterone production was observed at 0.1 and 0.5 μM BPS, accompanied by non-significant growth at 1, 10, and 25 μM BPS. However, a significant decrease was observed at the higher doses of BPS (50 - 100 μM). Testosterone levels increased significantly (P0.05) at 0.1 M BPS, accompanied by non-significant increase at 0.5, 10, or 25 μM BPF. However, at higher doses of BPF (50-100 μM), testosterone production was found to be significantly reduced. For a deeper understanding of bisphenols' endocrine disrupting activity, considerably more detailed and systematic research is required.

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## GENOTOXICITY EVALUATION OF THE FOOD ENZYMES PRIOR TO THEIR AUTHORISATION IN THE EUROPEAN UNION

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Animal, plant and microbial enzymes are widely used in food processing and preservation. A positive safety evaluation by the European Food Safety Authority (EFSA) is a prerequisite for their inclusion in the EU Community list. According to the new draft food enzyme guidance (currently under public consultation), genotoxicity tests are not always required for the safety assessment of certain food enzymes. Among all 94 scientific opinions on food enzymes published between May 2014 and April 2021, 70 are supported by genotoxicity tests. Applying today's standard, the authors showed that genotoxicity studies could be waived for 24 food enzymes. The genotoxic potential was determined by performing *in vitro* tests, covering both gene mutations and chromosomal aberrations (structural and numerical). Gene mutations were evaluated by Ames test (1 equivocal and 1 inconclusive out of 67 studies analysed, 3 studies were not considered) and chromosomal effects were evaluated by chromosomal aberration test (47 studies analysed, all negative) or micronucleus assay (20 studies analysed, all negative). Only 2 food enzymes required follow-up *in vivo* tests: *in vivo* micronucleus assay (2 cases) or a combination of an *in vivo* micronucleus assay and comet assay (1 case). Nearly all the food enzymes evaluated so far were not genotoxic, except 2 cases.

When genotoxicity tests are deemed necessary, the new draft food enzyme guidance considers food enzymes not as single chemicals, but rather mixtures in which not all of the components are well characterised. Therefore, the whole mixture should be tested firstly by a standard battery of the *in vitro* tests, comprising of the bacterial reverse mutation assay and the *in vitro* mammalian cell micronucleus test. Any confirmatory *in vivo* follow-up test needs to cover the same endpoint as the one which showed positive results *in vitro*. The recommended *in vivo* tests are the mammalian erythrocyte micronucleus test, the transgenic rodent cell gene mutation assay and the *in vivo* mammalian alkaline comet assay. Such tiered approach allows to cover in the most efficient way possible concern on genotoxicity that food enzymes might raise.

## ASSESSEMENT OF EYE IRRITATING POTENTIAL OF 59 CHEMICALS USING EPIOCULAR™ TIME-TO-TOXICITY PROTOCOLS

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Assessment of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The objective of the ALT4EI (ALTernatives for Eye Irritation) project was to confirm the testing strategy developed in the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project. These projects focussed on the development of tiered testing strategies for eye irritation assessment for all drivers of classification and evaluation of whether the test methods can discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category, No Cat) from chemicals requiring classification and labelling for Category 1 (Cat 1) and Category 2 (Cat 2). In the CON4EI project, a new testing strategy for EpiOcular ET-50 was developed, the sensitivity for predicting GHS Cat 1 and GHS Cat 2 chemicals was 73% and 64%, respectively and the very high specificity of 97% was maintained. None of the Cat 1 chemicals was underpredicted as GHS No Cat (1, 2). The goal of the ALT4EI project was to fill remaining data gaps and strengthen the data set.

A new set of 59 chemicals (41 liquids: (un)diluted, and 18 solids) was tested using the reconstructed human cornea-like epithelium (RhCE), EpiOcular, in two EpiOcular time-to-toxicity tests (Neat and Dilution ET-50 protocols). The set of chemicals contained 32 chemicals not requiring classification (No Cat) and 27 chemicals requiring classification (16 Cat 2 and 11 Cat 1). The chemicals were tested blinded in two independent runs by MatTek In Vitro Life Science Laboratories. In this study, a testing strategy to achieve optimal prediction for all three classes (No Cat, Cat 1 and Cat 2) that was developed in CON4EI project (which combines the most predictive time-points of both protocols and which tests liquids and solids separately) was used.
Using the CON4EI testing strategy and CON4EI developed prediction models for liquids and solids, we were able to identify correctly 63,6 % of the Cat 1 chemicals, 56,6 % of the Cat 2, and 76,6 % of No Cat chemicals. Reproducibility between both runs was 88,7 %. The combination of the EpiOcular time-to-toxicity neat and dilution protocols seem to be promising in an integrated testing strategy (ITS) for eye irritation assessment.

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## THE IMPACT OF DMSO ON CYTOCHROME P450 COMPLEX IN THE DEVELOPING CHICKEN LIVER

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Dimethyl sulfoxide (DMSO) is an organosulfur compound widely used as a solvent for small hydrophobic drug molecules. In this study, we documented the effects of DMSO in the developing chicken embryo at molecular level. The emphasis was placed on the developing chicken liver as the

main organ involved in the process of detoxification. In our study, 100% DMSO was administered subgerminally onto the eggshell membrane (*membrana papyracea*) at various volumes (5, 10, 15, 20, 25, 30, 35, and 50 µL) on 4th embryonic day (ED). At the molecular level, the cytochrome P450 complex (CYP) isoform's activities in relation to changes of CYP1A5, CYP3A37, and CYP3A80 gene expression was studied. The liver tissue for molecular analysis was collected on the 9th ED, followed by an extraction of total RNA and transcription to cDNA. Ubiquitin was used as endogenous control for calculating fold differences in RNA levels of cells treated vs not treated by DMSO using the $2^{-\Delta\Delta CT}$ method. DMSO treatment caused a significant different up-regulation of mRNA expression in all studied genes. The strongest effect of DMSO on gene expression in the form of up-regulation was observed in gene CYP1A5 (up to 3-fold), followed by CYP3A37 (up to 2-fold) and the lowest up-regulation was present in gene CYP3A80 (up to 1.5-fold). Down-regulation of gene expression in administered dose 5 µL in comparison to control samples with statistically significant level was observed in all studied genes. A significant down-regulation of all studied CYP isoform was detected after application of a DMSO dose of 5 µL. Our gene expression results revealed that even the lowest administered DMSO volume could affect hepatocytes at the molecular level causing down-regulation of cytochrome P450 complex (CYP1A5, CYP3A37, CYP3A80). These findings should be taken into account when the avian developing model is used for the testing of various substances dissolved in DMSO, mainly during its early stage of development. For DMSO use in a biological research, it is necessary to keep the working concentration as low as possible to not affect internal homeostasis of cells.

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## AN ADVERSE OUTCOME PATHWAY (AOP) BASED *IN VITRO* SCREENING OF NOVEL FLAME RETARDANTS INDUCED HEPATIC STEATOSIS

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The ban of polybrominated diphenyl ethers has led to the use of a wide range of novel flame retardants (nFRs) as a replacement. nFRs such as organophosphorus flame retardants (OPFRs) have been consistently de-

tected in increasing concentrations in the environment and human matrices. Evidence suggests that nFRs exposure may be associated with the disruption of endocrine system, which has been linked with the etiology of various metabolic diseases, including nonalcoholic fatty liver disease (NAFLD); however, the molecular mechanisms remain elusive.
NAFLD is a multifactorial disease characterized by the accumulation of lipids in the hepatocytes and involves multiple-hit pathogenesis, including exposure to occupational and environmental chemicals. Therefore, we aimed to screen and investigate the potential mechanisms for nFRs-induced hepatic steatosis in the human liver cells. In this study, we employed an *in vitro* bioassay toolbox to assess the key events (KEs) in the proposed AOP(s) for hepatic steatosis. We examined nine nFRs through *in vitro* assay and measured KEs such as lipid accumulation, mitochondrial dysfunction, gene expression, and *in silico* approach to identify the putative molecular initiating events (MIEs).
Our findings suggest that several tested nFRs induced lipid accumulation *in vitro* in human liver cells. The aromatic (aryl-) OPFRs, tricresyl phosphate (TMPP), triphenyl phosphate (TPHP), 2-ethylhexyl diphenyl phosphate (EHDPP), and the chlorinated OPFR, tris(1,3-dichloropropan-2-yl) phosphate (TDCIPP) induced the highest lipid accumulation by altering the expression of genes encoding for hepatic lipogenesis and mitochondrial dysfunction depicted by decreased cellular ATP production. Available data from ToxCast and *in silico* molecular docking suggests pregnane X receptor (PXR) and peroxisome proliferator-activated receptor-gamma (PPARγ) could be the potential MIEs. The study identifies several nFRs as potential risk factors for NAFLD and demonstrates the utility of an AOP-based strategy for screening and prioritizing chemicals and elucidating the molecular mechanisms of toxicity.

## DEVELOPMENT OF AN *IN VITRO* PROTOCOL FOR THE ASSESSMENT OF PHOTO-IRRITATION USING 3D RECONSTRUCTED HUMAN CORNEA-LIKE TISSUE MODELS

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In the recent years, the ISO standard 10993 for the Biocompatibility testing of Medical Devices implemented series of *in vitro* methods that help the safety assessors in the screening of potential health hazards of medi-

cal devices before conducting animal studies. Success has been achieved with development and validation of *in vitro* protocols for sub-cutaneous irritation testing that led to the release of the new ISO standard 10993-23 published in January 2021[1,2,3,4].

Building on the experience obtained in the above-mentioned project, an *in vitro* protocol combining the ocular irritation and photo-irritation endpoint has been developed using *in vitro* 3D reconstructed cornea-like tissue model (3D RHC). In the initial experiments we have evaluated the tolerance of the 3D RHC models towards an increasing dose of the UVA and visible light and selected a dose, that is sufficiently high to cause excitation of the tested compounds and in the same time is still non-toxic to the 3D model.

In the following experiment, we tested phototoxic benchmark Chlorpromazine and several materials representing medical devices and drugs used in ophthalmology to challenge the protocol. All materials were predicted correctly based on the viability and the proposed prediction model. In addition to the viability, we also evaluated IL1-α and TNF-α as markers of inflammation. The responses supported the *in vitro* predictions based on cytotoxicity.

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## THE REPREFRED SOCIETY

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The RepRefRed Society, "Gesellschaft zur Förderung von Alternativen Biomodellen", was founded on January 18, 2016 by researchers from the Medical University of Graz. With the support of researchers from the Medical Universities of Innsbruck and Vienna, the association aims to promote the implementation of the 3Rs (Replacement, Reduction and Refinement of animal experiments according to Russell and Burch 1959).
General goals of the RepRefRed Society are: a) sustainable provision of up-to-date information on the 3Rs; b) harmonization of further education and training concerning 3Rs methods; c) establishment of the Austrian 3R Center as a point of contact for researchers and the public to receive information about the 3Rs.
In order to achieve these goals, the association focuses on an active exchange of information among researchers through meetings and events. An increased transparency of protocols should lead not only to refinement of animal welfare but also to a reduction of the number of animals used in experiments. The network should also help to strengthen replacement by reporting on alternative approaches to animal testing.

## SUBLETHAL EFFECT OF PESTICIDES ON HONEYBEES AND ITS ROLE IN RISK ASSESSMENT SCHEME FOR PESTICIDES

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The risk assessment scheme for pesticides for western honeybees (*Apis mellifera* Linnaeus included) is based on "simple" measurement of the toxicity of active ingredient of pesticides. This relies on the determination of toxicity endpoints based on direct mortality induced by active ingredient of pesticides throughout the test (acute or chronic). The sublethal effects of pesticides on honeybees have been observed in several scientific

studies despite there was observed no statistically significant mortality during these trials. In our study we assessed potential harmful sublethal effect of commercial plant protection product containing spores of *Bacillus amyloliquefaciens* QST 713 on adult honeybees according to OECD 245 (2017). Caged honeybees fed 10 days with a sucrose solution (50% w/w) *ad libitum* spiked with a maximum application field rate of $4 \times 10^7$ colony-forming unit (CFU)/ml diet and its 1/10, showed decreased expression of genes encoding antimicrobial peptides in one/both treated groups in comparison with untreated control group. Significantly lower results of expression were detected in genes for Apidaecin, Hymenoptaecin and Defensin-1. The decrease in some immunity parameters observed in our study may potentially impair not only individual bee but also bee colony health and survival but this needs further study. After that we will be able to apply sublethal effects of pesticides on honeybees into the pesticide risk assessment scheme.

## SENSITIZATION POTENTIAL OF MEDICAL DEVICES DETECTED BY *IN VITRO* AND *IN VIVO* METHODS

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Medical devices have to be tested before marketing in accordance with ISO EN 10993-10 in order to avoid skin sensitization. This standard predominantly refers to the *in vivo* test; however, it doesn't exclude the use of *in vitro* methods, which have been sufficiently technically and scientifically validated for the purpose of medical devices testing. It is foreseen that due to the complexity of the sensitization endpoint, combination of several methods will be needed to address all key events occurring in the sensitization process. The objective of this follow-up study was to evaluate the sensitization potential of real samples of medical devices using a combination of *in vivo* (LLNA DA, OECD TG 442A), *in chemico* (DPRA, OECD

TG 442C) and *in vitro* (LuSens, OECD TG 442D) methods and to enhance testing strategy for the safety assessment of medical devices extracts. This limited study aims to optimize the use and preparation of extracts, with reference to our previous study (https://doi.org/10.14573/altex.2008142). A good agreement between in vitro and *in vivo* results was achieved regarding the absence of skin sensitization potential; however, discrepancies in positive classifications have been recorded. The mismatch between *in vitro* and *in vivo* results might be caused by specific response of the immune system of the living organism.

## 3R ACTIVITIES IN HUNGARY FOCUSING ON OUR ACTIONS

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The Hungarian legislation on the protection of animals used for scientific purposes entered into force in 2013 based on the EU Directive 2010/63. In Hungary, the Animal Welfare Bodies (AWB) task is advising on animal welfare issues focusing on the principles of 3Rs and increasing animal welfare. AWB is responsible for having well-trained, competent staff. In Hungary, five centers are entitled to give certification of competence approved by the Hungarian National Scientific Ethical Committee on Animal Experimentation. One of the educational centers is at the Semmelweis University. The main task of the educational centers is to increase the 3R-conscious' animal use in research.

**Aims:**

Collect and share information about 3Rs.
Determine the methods that promote animal welfare and 3Rs.
Build international contacts that help the development.

**Methods:**

Semmelweis University's Animal Welfare Body educates around 100 persons each year (technicians, assistants, graduated researchers) who carry on experiments on animals. We organize a course every year for maintaining the competence of certified persons.
We apply traditional and new methods in our education. In the theoretical part of education, we focus on the 3Rs from a different point of view (3Rs, statistics, analgesia and anesthesia, monitoring, ethics, health status, and pain monitoring) and alternative methods (*in vitro*, replacement, avian embryo).

We introduce the websites where the participants get information on 3Rs, especially alternative methods.
The statistical practice supports the reduction.
We involve the participant to apply actively their theoretical knowledge of 3Rs:
Everybody should take photos of the environmental enrichment in their animal facility.
The participants compile a short presentation about applying 3Rs in their research.
Placing a well-supplied ‚playground' in the lab, so the participants can follow up on the animal welfare all day.
We demonstrate the difference between an empty and enriched area on the behavior by applying open field observation.
Cooperation with companies helps the introduction of refinement methods.
While there is not a 3Rs center in Hungary the Animal Welfare Bodys are responsible for promoting 3Rs. We encouraging the participants to take part actively and summarize their knowledge of how to apply the basic principles in their research.

## AN *IN VITRO* HUMAN DIGESTION SIMULATION PLATFORM FOR FOOD-RELATED BIOACCESSIBILITY AND TRANSFORMATION TESTS

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A comprehensive platform including *in vitro* digestion simulation and associated analytical tools have been set up in our laboratory that can be utilized in food product development and toxicology. Regarding digestion simulation, the "Infogest" international consensus protocol is used that simulates adult oral, gastric and small intestinal digestive processes under standardised conditions. The analytical pillar includes instrumentation such as UHPLC-ESI-MS/MS, HPLC-ESI-QTOFMS, GC-FID and ICP-MS. This complex digestion simulation and associated analytical platform welcomes scientific cooperation and proposals in the field of among others studying i) interactions of macronutrients with each other, and with bioactive or toxic molecules, ii) transformations of phase II metabolites of toxins during digestion iii) accessibility of toxic molecules from food matrix and iv) effect of toxins on the digestive enzymes.

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## ABILITY OF THE GARDSKIN ASSAY TO PREDICT SKIN SENSITIZATION RESPONSE IN THE GUINEA PIG MAXIMIZATION TEST

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The preclinical safety assessment of medical devices typically involves an evaluation of the skin sensitization potential of the device. The GARDskin assay is being proposed as an *in vitro* alternative to the animal-based tests, Local Lymph Node Assay (LLNA) and Guinea Pig Maximization Test (GPMT), that are typically used to assess the skin sensitization potential of medical devices. The ability of the GARDskin assay to replace LLNA for prediction of skin sensitization response has been evaluated (e.g., Johansson et al., 2019) but since GARDskin has also been proposed as an alternative to the GPMT, it is important to compare the concordance of the prediction of the GARDskin assay with the *in vivo* response obtained in both of the animal-based tests. Based on the results of the GARDskin assay for 122 compounds, this *in vitro* assay shows a high concordance with the predicted results of the LLNA (87.5%); however,the concordance for results obtained in the GPMT is much lower (71.2%). The concordance of the GARDskin assay and the GPMT is impacted by the relatively high number of False Positive (FP) results (15 out of 73) compared to the FPs seen in the GARDskin vs. LLNA comparison (2 out of 80). The high number of FP in this comparison results from the inaccurate characterization of the human skin sensitization potential of these compounds by the GPMT. Therefore, the low concordance between the GARDskin assay and the GPMT is due largely to inaccurate predictions of human skin sensitization potential by the GPMT and not by shortcomings of the GARDskin assay. Notably, the GARDskin assay (88.7% accuracy) outperforms the GPMT (83.0% accuracy) in the ability to predict the human sensitization response of compounds in this dataset. The results of this project show that the GARDskin assay is able to predict skin sensitization potential with a level of accuracy that is equal to or exceeds that of the currently accepted animal-based tests, suggesting that the GARDskin assay can serve as a promising alternative to the GPMT and the LLNA as a means to assess the skin sensitization potential of medical devices.

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