

TCDD

TOXICOLOGIE

NUMMER 1
MAART 2020

SPECIAL THEME ANIMAL-FREE SAFETY

- MAAK KENNIS MET DE RIVM AFDELING VERNIËUWING TEST STRATEGIEËN
- DESIGNER DRUGS: LEGAL, BUT DEFINITELY NOT SAFE
- VALIDATION OF *IN VITRO* METHODS; TRAVELLING A LONG, WINDING AND BUMPY ROAD THAT IS LITTERED WITH DEEP HOLES
- BAMBOO CUPS: DANGEROUS AND NOT GREEN

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Editorial

With the beginning of a new year, we would like to invite you to think about what the future will bring us. Our field is rapidly moving away from animal testing towards human-relevant, mechanism-based innovative methods. This movement has gained momentum by the recent announcement of the US EPA that they will eliminate all mammalian study requests and funding by 2035. Also, within the Netherlands, we are committed to improve research into medicines, food safety and chemical risks without the need for laboratory animals. This commitment is exemplified by the Transition Programme for Innovation without the use of animals (Transitie Proefdiervrije Innovaties, TPI). A major challenge in this programme is the validation of *in vitro* methods. Traditionally, animals are used as the 'golden standard'. Recently, it has become increasingly apparent that there is large uncertainty in the outcomes of animal assays and that these golden standards are not as predictive for humans as we would like them to be. This calls for a new validation paradigm towards the acceptance of *in vitro* methods. And it calls for the training of a new

generation of toxicologists that help to speed up the development, validation and implementation of innovative methods.

In this TCDD, you will read about the Transition Programme for Innovation without the use of animals and the validation of *in vitro* methods. An important aspect in the transition is creating a dialogue between different stakeholders such as regulators, scientists and developers. This dialogue was successfully initiated at the International Pioneer-2-Policymaker conference. In addition, we give the floor to Dr. Janine Ezendam who recently became the head of the department of innovative testing strategies at RIVM to share their contributions towards new risk assessment and *in vitro* method development.

Especially for this XL edition, two of our TCDD editors describe the road towards validation of *in vitro* methods. This comprehensive article is for all of you that would like to read more about our "Animal free safety" theme.

You can also read an article on the renewed Postgraduate Education for Toxicologists (PET

courses). Dr. Karin van Ede and Dr. Elsa Antunes Fernandes Gaspar from KeyToxicology started as coordinators of the PET courses and help to train the new generation of Toxicologists.

We would like to take this opportunity to highlight this year's annual meeting: The (r) evolution of toxicological models – how to address safety in target species. At the annual meeting, we can continue our discussions on how to implement innovative models in regulatory risk assessment.

Finally, the winners of the Christmas puzzle and Christmas tree contest will be revealed. We hope you enjoy reading this TCDD!

On behalf of the editorial team,

Hedwig
Braakhuis



News from the board

With this year's Annual Meeting of the NVT only a few months away (June 10-11 in the Reehorst, Ede), the organizing committee is busy finalizing the final programme, which will be very exciting, see also <https://toxicologie.nl/meeting2020>. The theme of this year's meeting is The (r)evolution of toxicological models - How to address safety in target species. We are happy to announce that the registrations are open!! Early bird [registration](#) and [abstract submission](#) close on April 3, 2020. The board has also had some influence on the programme: we will take some time to explain the requirements for the NVT travel grant for PhDs, as well as hold our annual business meeting. To all toxicologists in the Netherlands, young and old: do not miss the event!

The Annual Meeting is not the only event organized by NVT this year. Also the sections have been busy organizing their spring symposia, including Occupational Toxicology (Circular Economy on March 12), Pharmaceutical Toxicology (Oncolytica, April 16), Environmental Toxicology (just held '50 Shades

of Filth' in January 2020), Risk Assessment (PFAS, April 7) and Teratology and Reproductive Toxicology (Obesity and Pregnancy, March 12). See the NVT website for further information on these relevant symposia. In addition, plans for the 16th IUTOX-EUROTOX 2022 World Conference in Maastricht on Sept 18-22, 2022 are well underway, see also <http://www.ict2022.com/>.

On behalf of the board,

Juliette Legler
President



SAVE THE DATES:

June 10-11, 2020
The Reehorst, Ede.

NVT meeting 2020

The (r)evolution of
toxicological models
– how to address safety
in target species.



Report of BEMS/DEMS meeting 15 October 2019

Similar to previous years, the Genotoxicology section of the NVT held its annual meeting together with the Belgian Environmental Mutagen Society on the 15th of October, which was entitled: 'CRISPR-CAS: the bright and dark side of human genome editing'. The symposium was hosted at the Leiden Bio Science Park.

The first lecture was given by **Prof. John van der Oost** from the Wageningen University. This presentation was an introductory lecture to the topic, explaining discovery and biology of CRISPR-Cas and its (potential) applications. The discovery that CRISPR-Cas is a heritable adaptive immune system of bacteria and archaea, based on RNA-guided DNA interference, initiated a revolution, including the exploration of the natural diversity of CRISPR-Cas classes and types. A range of applications in biotechnology and medicine have been developed from this.

The second lecture was given by **Prof. Niels Geijsen** from the Utrecht University discussing 'Gene editing with a grain of salt'. He elaborated on the clinical application of CRISPR/Cas9. Despite the exciting versatile clinical applications of this genome-editing tool, its safety and efficient delivery into patient cells remain to be established. Gene therapies based on viral delivery are costly and results in long term increase of unwanted off-target effects. Prof. Geijsen reported the development of a unique new method (iTOP) for the *in vivo* non-viral delivery of the

CRISPR/Cas9 protein into skeletal muscle, demonstrating this to be a simple, safe and consistently efficient tool to manipulate the skeletal muscle tissue *in vivo*.

Dr. Mick Fellows from AstraZeneca, UK continued with a lecture on the pre-clinical safety assessment of CRISPR therapeutics. The conversion of CRISPR/Cas9 technologies from laboratory tools to efficacious therapies has engendered a renewed belief that with the use of precise genome editing the curative value of gene therapy may finally be realized. Whilst the initial focus concerning the safety of CRISPR as a therapeutic was on potential off-target effects, more recently, the potential for on-target chromosome deletions and translocation has been highlighted, how can these be assessed and do they have oncogenic potential?

Following a networking lunch, the afternoon session was kicked off by **Dr. Katia Pauwels** from Sciensano, Belgium. She elaborated on the fact that CRISPR gene editing as an enabling technology is challenging in the

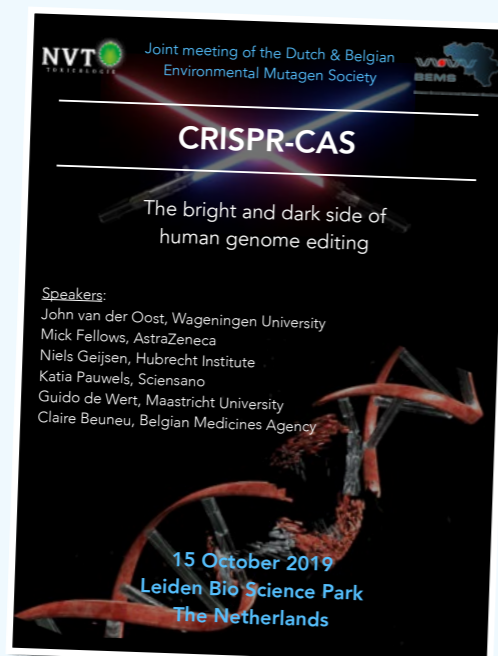
current genetically modified organism (GMO) regulatory landscape. Potential applications of genome editing include the development of animal models for research purposes, the generation of novel plant varieties and the expansion of the approaches taken for pest control. A ruling of the Court of Justice of the EU however, declared that organisms produced by directed mutagenesis techniques/methods should be considered genetically modified organisms within the meaning of the GMO Directive and subject to the relevant requirements, including a safety assessment with respect to their possible impact on human/animal health and the environment. Meeting the obligations of the GMO Directive implies cost- and labour-intensive pre-market evaluations and a long duration of the approval process.

Dr. Claire Beuneu from the Belgian federal agency for medicines and health products continued with a lecture on the risk assessment using CRISPR-Cas in clinical trial applications. The CRISPR-mediated genome editing technology is evolving rapidly and the first clinical trials have already started. Although CRISPR-Cas systems seem to present with amazing therapeutic potentials, many hurdles are still to be overcome, including the efficiency and specificity of the delivery method. The two most important safety concerns are the immunogenicity of the

different components of the CRISPR-Cas system and the potential off-target effects.

The closing lecture was held by **Prof. Guido de Wert** from the Maastricht University. He gave a challenging talk on the ethics of human germline genome editing (GGE). GGE is ethically controversial and legally prohibited in many countries, at least insofar as it regards reproductive (clinical) GGE. During his presentation he scrutinized the ethical and legal arguments for and against non-reproductive GGE (both fundamental research and pre-clinical safety studies) and possible reproductive GGE, making a distinction between, on the one hand, principled, deontological, arguments and, on the other hand, consequentialist arguments.

With approximately 60 participants from academia, industry and governmental organisations, the committee looks back on a successful meeting. ■



SECTION PHARMACEUTICAL TOXICOLOGY

11th Scientific Meeting of the Section Pharmaceutical Toxicology

We are delighted to invite you to the 11th Scientific Meeting of the Section Pharmaceutical Toxicology of the Dutch Society of Toxicology (NVT).

The Meeting is entitled **“Clinical and toxicological aspects of novel antitumor drugs”**. It will take place on the **16th of April 2020** and will be held in the Auditorium of the **O|2 Lab Building** at the VU Campus, De Boelelaan 1108, 1081 HZ **Amsterdam**.

We have composed an exciting programme with national key experts focusing on clinically and toxicologically relevant aspects of novel oncolytics, including the personalized applied clinical dosing, impact of transporters on kinetics as well as impact of the microbiome and the evaluation of these drugs from a regulatory point of view.

Speakers from (university) hospitals and government will share their perspectives.

The business meeting for members of the section Pharmaceutical Toxicology will take place before the start of the programme, where new board members will be elected.

Due to vacancies, colleagues interested in joining the board, are invited to apply; specially members working in the pharmaceutical industry. Application deadline is 15th of March 2020.

We hope the programme meets your interest and we welcome you on the 16th of April.

The registration fee is €15,- for master or PhD students, €25,- for members of the NVT and €35,- for nonmembers, due (in cash) at the registration desk on the day of the meeting. This includes coffee/tea, lunch and drinks at the concluding reception. **Registration deadline is 29th of March 2020**. You can register by sending an email to Dr. Jan Commandeur (j.n.m.commandeur@vu.nl) with the subject **“Pharmaceutical Toxicology Symposium 16APR2020”**. Please also indicate should you have specific dietary wishes.

Any cancellations after the 29th of March 2019 will unfortunately have to be charged for a cancellation fee of 100% of the registration fee. Early registration will ensure you can participate.

There will be a poster session during the lunch break. We therefore invite all those interested in presenting a poster to submit an abstract together with the registration to this event. Maximal poster size is A0; portrait-mode. A jury will invite the best 3 poster presenters to give a 3-slide PowerPoint presentation in the afternoon-session. The audience will subsequently decide by electronic voting, who is the winner and she/he will receive an **award** of 100 euros. **So, if you present a poster, you also need to prepare a 3-slide PowerPoint presentation!**

Best regards,

Section Pharmaceutical Toxicology: *Daan Touw (chair), Kris Siezen (treasurer), Yolanda Ponstein (secretary), Cathaline den Besten, Jan Commandeur, Sylvia Le Dévédec, Damiën van Berlo.*

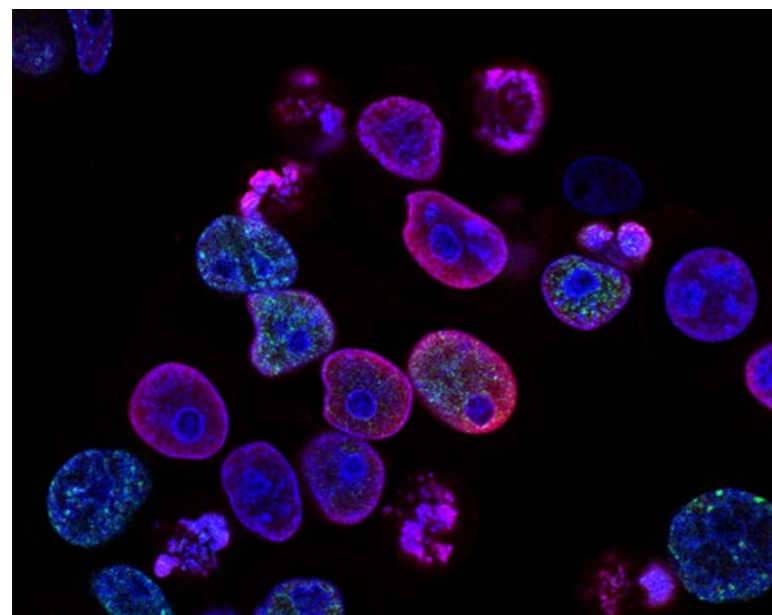


Photo by National Cancer Institute on Unsplash

Clinical and toxicological aspects of novel antitumor drugs

16th of April 2020

Organized by: the Section Pharmaceutical Toxicology of the Dutch Society of Toxicology (NVT)

Location: Auditorium of the O| Lab Building at the VU Campus, De Boelelaan 1108, 1081 HZ Amsterdam

| Meeting programme | | |
|----------------------|---|--|
| From 09.30 h | Registration with Coffee and Tea Set-up of posters | |
| 10.00-10.30 h | Business meeting (Huishoudelijke vergadering) for members of the Section Pharmaceutical Toxicology (election new board members) | |
| 10.30-10.35 h | Opening Scientific Meeting | By the chair of the Section Pharmaceutical Toxicology |
| 10.35-11.10 h | Dr N. Steeghs (NKI / DPOG) | General introduction new developments on oncolytics |
| 11.10-11.45 h | Dr. N. van Erp (Radboud UMC / DPOG) | Personalized dosing instead of Flat dosing of targeted oncolytics |
| 11.45-12.20 h | Dr. C. Herberts (CBG) | Evaluation new oncolytics from a regulatory point of view |
| 12.20-13.40 h | LUNCH – Poster exhibition – Network opportunity (all in the same area) | |
| 13.45-14.20 h | Dr. A. Schinkel (NKI) | Impact of transporters on the pharmacokinetics of novel anticancer drugs |
| 14.20-14.55 h | Dr. B. Venhuis (RIVM) | Oncolytics in surface water |
| 14.55-15.30 h | Dr. J. de Haan (UMCG) | The microbiome & oncolytics |
| 15.30-15.50 h | Plenary presentations of the best 3 posters (5 minutes per poster) & election best poster (by the audience) | |
| 15.50-15.55 h | Closure | |
| From 16.00 h | Drinks | |



SECTION RISK ASSESSMENT

Invitation Spring symposium: 'Everything you wanted to know about PFAS'

7th of April 2020 (13.00 – 17.00 h)

Location: NVWA, Graadt van Roggenweg 400, Utrecht

Meeting programme

| | |
|-------------|---|
| 13.00-13.20 | Registration |
| 13.20-13.30 | Welcome |
| 13.30-13.50 | Introduction – Prof. dr. Annemarie van Wezel (UVA) |
| 13.50-14.20 | Determination of PFAS limits in soil – Dr. Arjen Wintersen & Dr. Piet Otte (RIVM) |
| 14.20-14.50 | Policy consequences |
| 14.50-15.10 | Coffee break |
| 15.10-15.30 | From soil to (drinking) water – Dr. Frederic Béen (KWR) |
| 15.30-15.50 | From water to livestock to consumers – Dr. Krista Bouma & Dr. Jacqueline Steenbergen – Biesterbos (NVWA) |
| 15.50-16.30 | Discussion & wrap-up |
| 16.30-17.30 | Drinks |



Source: NOS

You can register for this meeting by sending an email to Monique Nagtegaal (m.nagtegaal@nvwa.nl). Please state "Registration NVT spring symposium – 7 April" in the title of the email and report your name and affiliation in the email itself.

The Transition Away from Animal Testing

TCDD: Will it be feasible in the course of the coming 15 years to exclude animal testing for regulatory purposes without affecting safety?

Cyrille Krul: This question is difficult to answer and as you may expect from a scientist, my answer would be: it depends. It depends whether we will be able to make optimal use of the recent developments e.g. in data science and advanced *in vitro* models. These new technological opportunities can accelerate the transition to animal free testing. If we combine these new technologies, we might be able to better understand the (human) biological mechanisms and improve the prediction of the adverse outcomes. However, we also have to realise that technological and scientific development is only one of the drivers to move away from animal testing, but there are more barriers to overcome before animal testing for regulatory purpose will become redundant.

We have to carefully look for both drivers and barriers in this process and the role of the different stakeholders involved. There are many different parties involved, such as academic and applied researchers, contract research organisations, technology providers, pharma and chemical companies, government, regulators, patients, citizens, non-governmental organisations etc. If these organisations can collaborate from the early start and

work together on a collaborative research agenda, we will be able to develop new testing strategies, based on data obtained from human-relevant models.

One of the major challenges is that regulatory safety testing is globally regulated and therefore we need to involve (inter)national regulators as well, e.g. to discuss the acceptance criteria of new methods. Most larger companies are producing worldwide. Thus, regulatory frameworks and testing strategies should be globally accepted and as a result we need globally harmonized legislation. And we know that this can be very time consuming and is a matter of longevity.

In first instance, we need pioneers, scientists that are motivated and willing to explore new approaches, to learn from those experiments and to share their experience with other organisations. Communication and sharing data is key in the transition. Early adopters can inspire other colleagues in the field, or even outside their own scientific area. Thus, let's not forget the importance of education. Not only initial education, but also training and hands-on workshops to explore new technologies and risk assessment methodologies later on in your career. As with every transition, we know that the way forward will for sure not be a paved and straight path. Therefore, we need longer term commitment from researchers, but also from funding organisations, to give researchers



Dr. Cyrille A.M. Krul

Professor Innovative Testing in Life Sciences & Chemistry

Director Research Center Healthy and Sustainable Living

University of Applied Sciences Utrecht (Hogeschool Utrecht)

the opportunity to investigate all the new developments. We also need to collaborate with scientists from other fields, previously not involved in biomedical research, such as computer science. Understanding each other and developing common language simply takes time.

Furthermore we should facilitate researchers, e.g. by providing them access to vital human tissue. If vital (non-preserved, fresh) tissues can be made available to all researchers in the Netherlands, also to those researchers not working in academic hospitals, they can develop more advanced human models. An initiative called Vital Tissue (www.vitaltissue.nl) has recently started with eight different organisations to do a feasibility study. →

And the last driver we could think about is to reward people. In line with the access to medicines index a benchmark tool named Beyond Animal Testing-index will be developed to reward organisations for their effort in the transition to animal free testing. Such a tool can help to show what is already possible and where effort is still needed.

I am a positive person and believe that animal experiments will someday become redundant. But the questions is: when? So much more seems to be possible compared with ten years ago, however nobody can predict how fast it will happen. In the end, only people can make the difference! ■

Safety Testing: Evolution vs. Revolution

TCDD: Will it be feasible in the course of the coming 15 years to do away with animal testing for regulatory purposes without affecting safety?

Anne Kienhuis: Regulatory safety testing is in transition. Since decades, a lot of time and effort has been invested in the reduction, refinement and replacement (3Rs) of animal studies. This has led to a toolbox with various animal-free (*in silico*, *in vitro*) toxicity methods intended for use in regulatory safety assessment, but a complete replacement of animal tests is not yet possible. From a safety-first perspective, we should carefully analyze the value of each new concept and technology for their application in regulatory safety assessment, depending on the needs of each stakeholder in the process (e.g. academia, industry, government, regulatory authorities, society).

As such, we cannot speculate about the pace of the transition and the feasibility to ban animal testing within the next 15 years for regulatory purposes. We do believe that the transition to safety assessment without animal testing can be accelerated by interdisciplinary and multi-stakeholder collaboration, thereby simultaneously investing in two approaches: the evolutionary and the revolutionary approach.



Anne Kienhuis

Senior scientist at the Department for Innovative Testing Strategies, Centre for Health Protection

National Institute for Public Health and The Environment (RIVM)

In the **evolutionary approach**, regulatory-required animal studies are gradually replaced by (sets of) animal-free methods. Previous research has shown that there are generally no legal barriers for the use of animal-free methods in regulatory frameworks. For some toxicity endpoints, such as skin sensitization, genotoxicity, irritation and corrosion, several animal-free methods are described OECD test guidelines and accepted by regulatory agencies. Numerous drivers and barriers have been identified that are influenced by the various stakeholders involved in safety assessment, determining the pace in which *in vitro* methods are accepted and →



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implemented in regulatory frameworks. These include the currently time-consuming and expensive international validation processes for animal-free methods and strategies. Furthermore, complete replacement of animal studies is hampered by the fact that the toolbox of animal-free methods does not deliver all the necessary information, nor cover all the legally required toxicity endpoints perform human safety assessment yet.

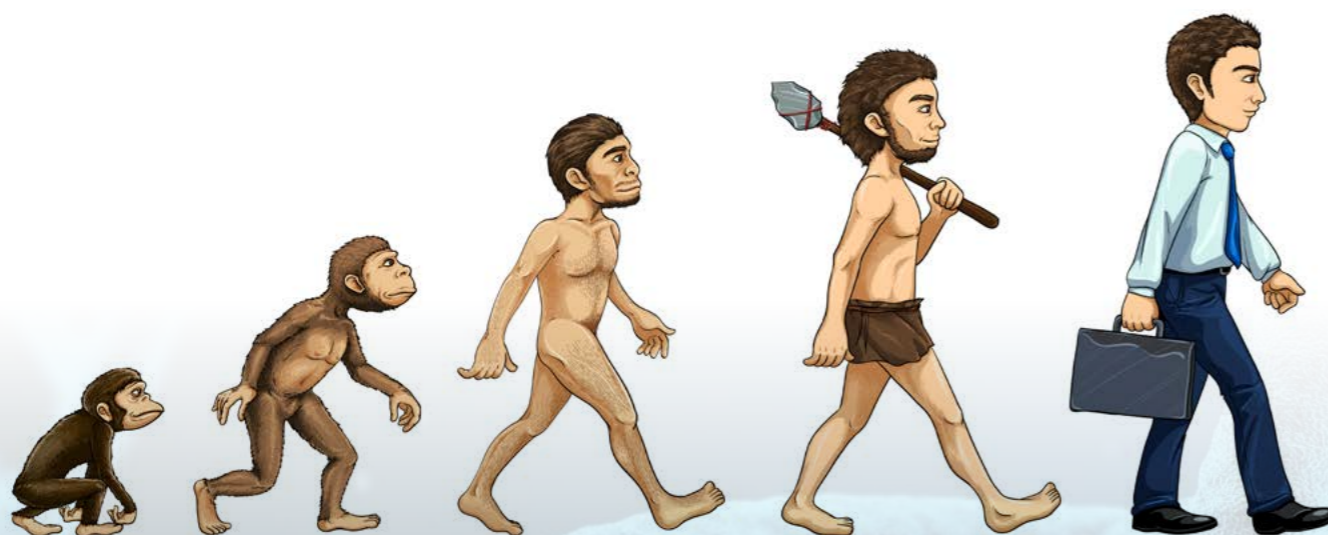
The **revolutionary approach**, on the other hand, moves away from the animal study as the gold standard and proposes human biology and physiology as the basis for a paradigm shift in human safety assessment. This approach is conceptual, independent of current legislation, and allows to investigate emerging concepts, such as the virtual human, and the integration of innovative technologies, such as artificial intelligence and advanced *in vitro* models (e.g., organ-on-a-chip) for application

in safety assessment. A key factor for success for this approach is collaboration between the various disciplines behind these innovations, such as disease modeling and data science, to see how these can innovate toxicology testing and improve human safety assessment. The revolutionary approach may offer opportunities to investigate human-relevant mechanisms and adverse effects that are currently insufficiently covered, such as neurodegenerative diseases.

In conclusion, the evolutionary approach will increasingly rely on combinations of *in vitro* methods, gradually replacing animal toxicity studies. A true paradigm shift in animal-free safety assessment would be to move away from the animal study as the gold standard and use human biology as the new reference instead. In this way, we can improve safety testing and at the same time reduce animal testing. ■

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vs.



The International Pioneer-2-Policymaker conference on the transition towards animal-free innovation

(Utrecht Science Park, 27th - 29th of November)

Highlighting the Organizing Trust & Confidence and Business Development Opportunities sessions

The international Pioneer-2-Policymaker conference was held at Utrecht Science Park (27-29 November); hosting was made possible by Utrecht Life Sciences and the Utrecht-Advanced *in Vitro* Models hub (U-AIM). Over 150 representatives from academia, research funders, large-scale companies, start-ups, government, regulators and NGOs were received. The delegates originated from 16 different countries. The conference was organized by the Transition Programme for Innovation without the use of animals (TPI) spearheaded by the Ministry of Agriculture, Nature and Food Quality (LNV); other ministries involved were EZK, VWS, I&W and OCW. TPI partners included RIVM, Proefdiervrij, SGF, ZonMw, VSNU (the 14 Dutch universities), NFU and Topsector Life Sciences & Health. The meeting was moderated and co-created by the experienced discussion leaders from Meneer de Leeuw.

The principal aims of the conference were to 1) present "the Dutch way" of accelerating the transition to international parties and 2) to create a true dialogue between researchers, regulators and developers.

A varied programme was put together with highlights including a presentation from Carola Schouten (Minister of LNV), keynote lectures from Christine Mummery

(Leiden UMC) and Adrien Roth (Roche) and a highly dynamic carousel session in which a number of Dutch initiatives to stimulate animal-free research (including *Vital Tissue*, U-AIM, hDMT and the *Helpathon*) were pitched and briefly discussed. International participants also were given the opportunity to present policies and practices from their home country aimed towards the stimulation of animal-free testing methods.

To stimulate dialogue between stakeholders and due to the sensitivity of the topic, a "whispering" format was adopted; no audio recordings were made and participants were requested not to quote each other outside of the conference environment. This means that a detailed conference report will not be available; information will be shared among conference participants only. For this reason, we chose to zoom in on the two sessions that the authors had the pleasure of hosting: "*Organizing Trust & Confidence*" session (Sue) and the "*Business Development Opportunities*" session (Damiën) and how we personally experienced them, including lessons learnt.

One of the highlights of the conference was the ceremony for the Willy van Heumen award to Remco Westerink, head of the Neurotoxicology group at Utrecht

By Sue Gibbs (Amsterdam UMC, ACTA) and Damiën van Berlo (U-AIM hub, Utrecht University)



University; he received this award (including €15k) for the work performed at his group on an animal-free brain model called the microelectrode array. This system involves the culture of human stem cells that grow into brain-like structures on special microtiter plates with electrodes attached to the bottom. With this system, intercellular signals and the effect of foreign stimuli on these signals can be measured, which has great value for neurotoxicity testing.



Willy van Heumen award winner: Dr. Remco Westerink

Organizing Trust & Confidence session, hosted by Sue and Anne Kienhuis (RIVM): this session was designed to attract an interdisciplinary team of participants from industry, academia and regulatory bodies. The moderator, Jantine Wijnja (Meneer de Leeuw) led the whispering session together with the hosts. The aim of the session was to



uncover new ways of thinking and acting to organize trust and confidence for emerging human based research methods. We invited participants to be open to each other's knowledge and perspective; daring to listen and possibly even to play with silence. Daring to speak their minds honestly and searchingly, leaving the comfort of familiar opinions behind. In this way, the first steps were taken to gain *Trust and Confidence* in each other.

We all want our pharmaceuticals and chemicals to be safe for human exposure and to ensure that we have built regulatory frameworks, including methods to test for safety and efficacy or mode-of-action of our products. Many of these test methods were developed during the time that animal models were considered to be the best predictors of human safety. Tremendous progress has been made since then, using animal-free knowledge and tools, but these don't 'fit' within the existing regulatory framework. On the top of the list of questions asked and discussed were: how can we jointly organize trust and confidence in emerging *in vitro* and *in silico* tools? When is a tool good enough? How can we create room for innovation? What do we need to truly and fully rely on the outcomes of animal-free tools or models? What can we, as participants in this session, do to create movement and affect change? These very important questions were discussed in a safe environment enabling everyone to openly say where their problems and bottlenecks were, and also enabling participants to help and advise their neighbour by offering specialist expertise. In one case it was an academic who had no idea how to contact regulatory people to ask advice when starting to develop

their new research model, in another case it was industry and regulatory representatives seeking easier ways to communicate their specific needs for regulatory acceptance. The relevance of validating emerging methods using data from regulatory accepted animal models was discussed; participants agreed that it would be better to validate such methods using human data, but how do we access those data? In academic research, new tests and models can be readily implemented (no regulations apply), but we still need to find effective ways to share the knowledge on useful non-animal models so that they can be implemented. Moreover, since chemical and drug industries develop animal-free product safety tests, some wondered if such developments could be better communicated to the wider scientific community and to regulators.



At the end of the session, participants had made contacts for following up, in small groups, after the conference. Participants who wanted to be actively involved with the TPI sessions at the WC11 also came forward.

Business Development Opportunities session, hosted by Damiën and moderated by Jan de Dood (Meneer de Leeuw): this session was designed by an interdisciplinary team consisting of Martje Fentener van Vlissingen (Erasmus MC), Merel Ritskes-Hoitinga (Radboud UMC), Debby Weijers (Proefdiervrij), Nico van Meeteren (TopSector LS&H), Jan de Dood (Meneer de Leeuw), Anke Sikkema (TPI) and Damiën van Berlo (UU/U-AIM). It was mainly targeted at companies (from start-up to big pharma). With this session we attempted to address one of the most relevant questions for the advancement of innovative *in vitro*/animal-free science: how can we attract interest from investors/companies? Their involvement is essential to bridge the gap between an academic concept model and a fully characterized and validated model, ready for implementation in drug discovery or for regulatory testing. If we want to replace animal tests, we need the latter, not the former; in fact this also touches on the topic of the parallel session (Building Trust & Confidence), because the availability of well-validated *in vitro* models will greatly boost the confidence



among regulators in the replacement of animal tests without posing risks to the general population.

During the BDO session, four very different animal-free models were pitched to the audience; intestinal/airway organoids (Jeffrey Beekman, UMC), bio-printed liver (Bart Spee, UU), the Polycystic Kidney Disease array (Ocello, Leiden) and precision-cut tissue slices (Medical Innovation Xccelerator, Groningen). After the pitches, the models were discussed in parallel roundtable discussions, focusing on what would be needed to move models forward in terms of development. Afterwards, the model pitchers reflected on the feedback received, while Marijn Vlaming (Charles River Laboratories) provided the CRO perspective on the presented models. The model presenters received useful feedback; independent of technology readiness level, it is crucial to approach potential end users early on and take their input into account. Also, the session showed the great value of scientific entrepreneurship for connecting researchers with small-to-medium enterprises and larger companies; a start-up will have knowledge of the market, the competition, the purpose



and the unique selling point(s) of a certain *in vitro* model. Stimulating researchers to consider launching a start-up to advance their promising models would help accelerate animal-free innovation. When the session finished, participants were invited to have a look at two *in vitro*/ex vivo demonstrations (a microtome to make precision tissue slices was demonstrated by Medical Innovation Xccelerator and there was a multi-Organ-on-Chip system demonstration by U-AIM).

Conclusion: Have the main goals been achieved? Firstly, the Dutch way of moving towards animal-free test methods was well received by international participants. An impression of international responses can be found on the TPI website, including video clips with brief interviews:

<https://www.transitieproefdiervrijinnovatie.nl/english/what-tpi-does-and-supports/international-conference>

<https://www.transitieproefdiervrijinnovatie.nl/documenten/videos/20/1/9/looking-beyond-borders>

<https://www.transitieproefdiervrijinnovatie.nl/documenten/videos/20/1/29/invisible-walls>

Secondly, true dialogue between the different stakeholders was certainly achieved. Because of the relative safety of the created conference environment, all major stakeholders were represented and took part in the discussions. The design of the conference (whispering conference, World Café format, high level of interactivity) by Meneer de Leeuw certainly contributed to this, but the highly involved participants themselves deserve a large portion of the credit. “Thank you” to all participants for your contributions, on to WC11! ■

The Use of Animals in Toxicity Tests: A European Perspective

Statement to the TCDD from the European Commission

In the EU, the use of animals is forbidden when non-animal alternatives are available, and the use of animals is only allowed when alternatives do not yet exist.

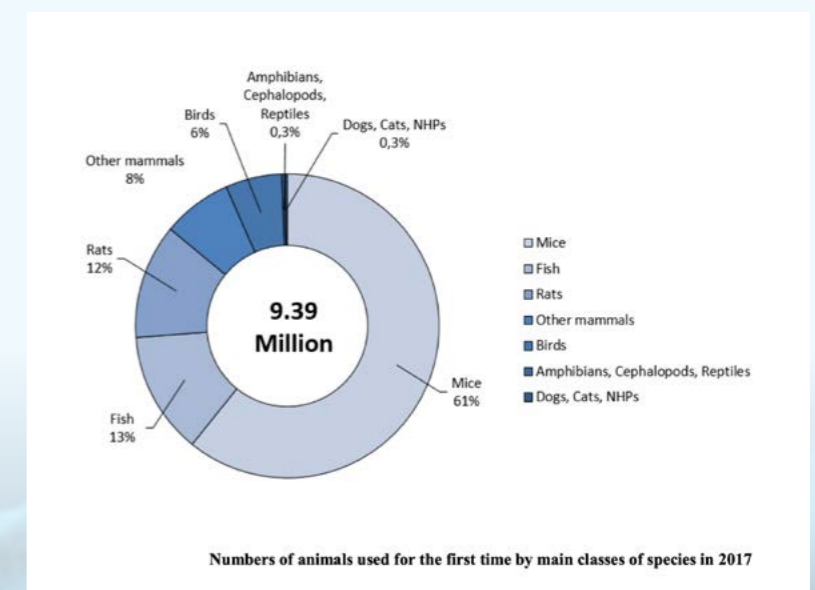
Over the last decades, technological advances have revolutionised biomedical research. Major breakthroughs include the development of alternative tests based on cell and tissue cultures, and computational methods that reduce the need for testing animals.

However, many complex physiological and toxicological processes and effects cannot yet be adequately modelled or assessed by non-animal methods, so some animal studies are still needed to advance research and to safeguard human, animal and environmental health.

The Commission will continue to support accelerating progress in the area of replacing, reducing and refining the use of animals in research and testing. We are doing this through **focused activities addressing specific areas of animal use, proper implementation of the legislation and activities on education and training, and knowledge sharing**. The Commission will also continue to invest in the development and validation of alternative approaches.

Previous attempts to setting an EU target, for example in the 5th EAP, have shown that generic, over-all targets are not effective. Animal use is influenced by several factors, such as the availability of research funds, economic trends, outbreak of epidemics, breakthroughs in alternative methods etc.

The [EU statistical report](#), which was recently published, allows us to better identify areas where we can advance most effectively towards the ultimate aim of replacing all use of animals. ■



Maak kennis met de RIVM afdeling Vernieuwing Test Strategieën

Waar “animal-free safety” een belangrijk thema is

Sinds 1 januari 2020 ben ik hoofd van de afdeling Vernieuwing Teststrategieën (VTS) van het Centrum Gezondheidsbescherming van het RIVM. Deze afdeling is voor mij niet nieuw, ik ben er al flink wat jaren werkzaam als immuuntoxicoloog. De afdeling bestaat uit 25 mensen: wetenschappelijk onderzoekers, analisten en promovendi.

PROFIEL AFDELING

In de afdeling VTS ondersteunen we het beleid van verschillende ministeries en toezichthouders op het gebied van risico's van chemische stoffen, nanotechnologie en medische technologie en over



RIVM afdeling Vernieuwing Test Strategieën

nieuwe (proefdiervrije) testen en strategieën om deze risico's te kunnen schatten. We voeren zelf onderzoek uit om wetenschappelijke kennis te krijgen op bovengenoemde thema's. Ook kijken we vooruit om nieuwe technologieën, proefdiervrije innovaties en wetenschappelijke inzichten te signaleren. Aandachtsgebieden zijn reproductietoxicologie, (ontwikkelings)-neurotoxicologie, immunotoxicologie, inhalatietoxicologie, hormoonverstoring, genetische toxicologie en carcinogenese.

VERNIEUWING RISICOBEOORDELING

De rode draad van de afdeling is vernieuwing van de risicobeoordeling, waarbij we zoveel mogelijk gebruik willen maken van innovatieve proefdiervrije methoden.

Het idee hierachter is dat we de risico's beoordelen op basis van de humane biologie en hoe blootstelling aan stoffen schadelijke effecten hierop kunnen hebben. Momenteel werken we aan twee scenario's: de evolutionaire en de revolutionaire aanpak (meer info hierover in het stuk van Anne Kienhuis elders in deze TCDD). We



Janine Ezendam

dragen bij aan diverse internationale initiatieven waarin we deze nieuwe benadering samen met anderen vorm geven, waarbij we werken aan belangrijke bouwstenen van de nieuwe risicobeoordeling. Het gaat om zowel het toepassen van concepten als Adverse Outcome Pathways (AOPs) (Luijten et al., 2020) en 'ontologies' (Staal et al., 2017), als ook proefdiervrije innovaties waarmee verstoringen van biologische processen kunnen worden gemeten. We houden een vinger aan de pols ten aanzien van opkomende proefdiervrije innovaties, zoals organ-on-a-chip, en hun eventuele meerwaarde voor de risicobeoordeling (Heringa et al., 2019). Andere aandachtsgebieden zijn machine learning en artificial intelligence. De stip op de horizon is de 'virtual human', een computerplatform waarin de veiligheid van stoffen wordt geëvalueerd op basis van de humane biologie (Piersma et al., 2019).

LABONDERZOEK

Het labonderzoek van de afdeling is vooral gericht op ontwikkeling van alternatieve methoden: →

Zebravisembryo's gebruiken we voor onderzoek naar de toxische effecten van mengselblootstelling (Zoupa et al., 2019), voor ontwikkelingsneurotoxicologie en voor reproductietoxicologie.

In vitro longmodellen zetten we in voor onderzoek naar de toxische effecten van stoffen die we inademen, zoals nanomaterialen. Hierbij maken we gebruik van de air liquid interface (ALI) om de cellen via de lucht bloot te stellen.

Embryonale stamcellen (humaan en muis) gebruiken we in ons onderzoek naar ontwikkelingstoxiciteit. We bestuderen de effecten van chemische stoffen op de ontwikkeling van neurale cellen (De Leeuw et al., 2020) en hartspiercellen (Mennen et al., 2019).

In vitro modellen voor immunotoxicologie en immunomodulatie gebruiken we voor verschillende internationale projecten: het IMI project VAC2VAC waarin proefdiervrije methoden worden ontwikkeld voor humane en veterinaire vaccins, het EU project REFINE gericht op de regulatoire behoeftes rondom testen van nanomedicines (Giannakou et al., 2016) en het ZonMw project gericht op het testen van de immunotoxiciteit van microplastics.

Medische hulpmiddelen. Binnen het RIVM Centrum Gezondheidsbescherming werken we in verschillende afdelingen aan de veiligheid van (hoog-risico) medische hulpmiddelen, zoals fillers, borstimplantaten en bekkenbodematjes (<https://www.rivm.nl/medische-hulpmiddelen>). In onze afdeling richten we ons op de ontwikkeling van *in vitro* testen waarmee kan worden

aangetoond of een bepaald medisch hulpmiddel veilig is. Hierbij kijken we o.a. naar mechanotoxicologie, omdat naast chemische ook mechanische prikkels rondom een implantaat tot ongewenste gezondheidseffecten kunnen leiden.

VALIDATIE, ACCEPTATIE EN IMPLEMENTATIE VAN NIEUWE TESTEN IN DE REGELGEVING

Naast het onderzoek op het lab, werken we ook aan de ontwikkeling van teststrategieën waarin verschillende testen worden gecombineerd, bijvoorbeeld voor genetische toxiciteit en huid sensibilisatie. Dit doen we in nauwe samenwerking met risicobeoordelaars van het RIVM die betrokken zijn bij de veiligheidsbeoordeling van chemische stoffen (bijv. REACH, EFSA) en voor geneesmiddelen met de beoordelaars van het College ter Beoordeling van Geneesmiddelen (CBG).

Validatie is een belangrijke schakel om nieuwe testen geaccepteerd te krijgen. De huidige procedure is ingericht voor individuele testen. In de toekomst zullen we vaker met combinaties van testen te maken krijgen; hier is waarschijnlijk een andere procedure voor nodig. Rondom dit onderwerp hebben we samen met de Duitse BfR, het 'German Federal Institute for Risk Assessment' twee workshops georganiseerd (Burgdorf et al., 2019).

VAN 3V BELEID NAAR TRANSITIE PROEFDIERVRIJE INNOVATIES(TPI)

Het RIVM is al decennia actief op het gebied van onderzoek en beleidsadviesing over de 3Vs. We werken op dit thema veel samen met kennisinstellingen, universiteiten en bedrijven binnen en buiten Nederland. We hebben al geruime tijd een mooie samenwerking

met het IRAS van de Universiteit Utrecht, waar Aldert Piersma sinds 2007 bijzonder hoogleraar alternatieven voor dierproeven is. Deze leerstoel is sinds 2016 deels gefinancierd door de Stichting Proefdiervrij. Het 3V beleid heeft een nieuwe impuls gekregen door de Transitie Proefdiervrije Innovaties (TPI), ingezet door het ministerie van LNV (www.transitieproefdiervrijeinnovatie.nl). Het RIVM draagt bij aan dit initiatief. De aanleiding voor TPI is de ambitie van LNV om Nederland om ervoor te zorgen dat Nederland internationaal voorloper is in proefdiervrije innovatie. Het RIVM heeft in 2018 de Agenda Proefdiervrije innovaties in het regulatoire veld uitgebracht (www.rivm.nl/agenda-PIRV), hierin worden de stappen beschreven die nodig zijn om deze transitie naar een proefdiervrije risicobeoordeling te maken.

Zoals jullie kunnen lezen zijn we een enthousiast en proactief team dat zich richt op onderzoek en beleidsadviesing op diverse maatschappelijke relevante thema's rondom het thema animal-free safety. Samenwerking is in ons oogpunt cruciaal. De transitie naar een proefdiervrije risicobeoordeling kan alleen bereikt worden door inter- en multidisciplinaire samenwerking, door een transparante en heldere communicatie en door het betrekken van relevante stakeholders.

Meer informatie over wat het RIVM doet op het gebied van alternatieven voor dierproeven is te vinden op: www.rivm.nl/vervangen-verminderen-en-verfijnen-van-dierproeven. Wil je op de hoogte blijven van wat er speelt op het gebied van 3V's, proefdiervrije innovaties in het regulatoire veld: abonneer je dan op onze nieuwsbrief via www.rivm.nl/3rs-quarterly. →

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Validation of *in vitro* methods;

Travelling a long, winding and bumpy road that is littered with deep holes

By Damiën van Berlo and Jasper Woutersen

The Dutch government is very ambitious when it comes to replacement of animal testing by *in vitro*, *ex vivo* and/or *in silico* methods or combinations of these. In 2025, the Netherlands wants to be a forerunner in animal-free innovations. Other countries have also communicated ambitions regarding animal-free testing. Last September, the US Environmental Protection Agency (US EPA) has communicated that animal testing is to be phased out by 2035 and replaced by “cutting-edge, ethically sound science”. The kind heart of

Donald Trump? The EPA has included an escape however: after 2035, any tests or funds for studies involving animals such as mice would require the approval of the EPA administrator. So, on a case-by-case basis, animal testing would still be allowed. By 2025, funding of animal studies is to be reduced by 30%. Cutting costs on animal studies might not be the most convincing way to show dedication to animal-free testing; increasing funding for *in vitro* innovations would achieve more in that respect.

With this piece we would like to shine a light on the process of validation of *in vitro* methods that could be implemented to replace animal testing. According to the Cambridge Dictionary, validation can be defined as, “the act or process of making something officially or

legally acceptable or approved.”. For the purpose of this article, something is an *in vitro* test method. This sounds simple enough, some might even assume that all methods published in scientific journals should be valid, otherwise publication would be rejected. Would we therefore not have lots of valid and accurate *in vitro* test methods? Certainly, we have lots of promising early concept models. But test methods validated for application in regulatory testing procedures? →

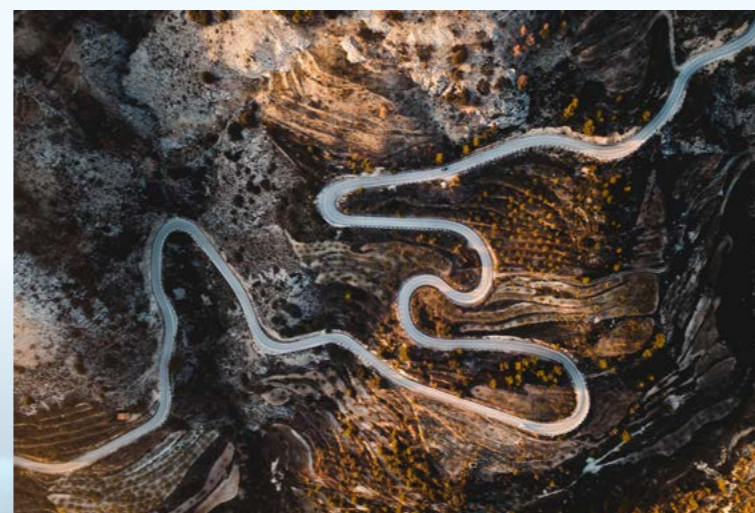


Photo by Jack Anstey on Unsplash, winding road



Photo by Jannes Glas on Unsplash, winding and bumpy road

Unfortunately, these are scarce. Why is this so difficult? An RIVM report investigating legal barriers for the use of animal-free alternative test methods (Heringa et al. 2014) shows that it's not legal, but rather practical boundaries that obstruct the implementation of animal-free testing procedures. In fact, there is a lack of tests because they are either unsuitable or insufficiently validated. Where are such validation tests performed? What regulations do we have and who decides whether a test is validated or not? We will attempt to answer such questions in this small and far from complete summary.

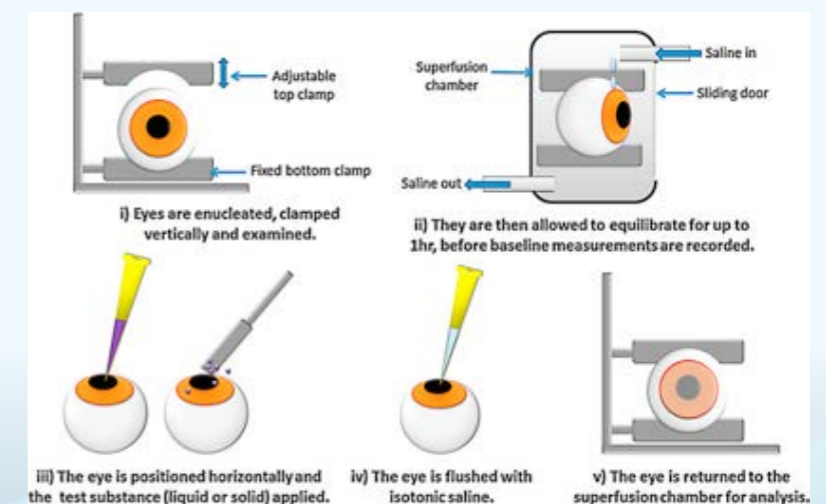
Validated? For what, exactly?

For instance, if we want to replace animal models by animal-free innovative test methods, when do we consider the model to be validated? And for what purpose? As a full replacement of animal testing or just as screening method? In most cases, existing *in*

vitro methods that can be used for regulatory testing are validated for use in pre-screening (e.g. tests for genetic toxicity) or for use with specific categories of substances (e.g. Isolated Chicken Eye test). The existing *in vitro* test guidelines are mainly focused on four areas: skin corrosion and irritation, eye irritation, skin sensitization and genetic toxicity. But there are only two areas where the *in vitro* methods can completely replace the need for animal testing, i.e. 1)

skin corrosion and irritation and 2) eye irritation. These areas can be considered as the "simpler" endpoints in human toxicology. To assess skin irritation (OECD test guideline 439), reconstructed human skin is used to test if substances can induce significant cell damage. Additionally, models have also been developed that use the eyes of slaughterhouse animals; cows in case of the Bovine Cornea Opacity/Permeability (BCOP) assay (OECD test guideline 437) and chickens in case of the Isolated Chicken Eye (ICE) test (OECD test guideline 438). When a substance tests positive in the BCOP or ICE (or in the Short Time Exposure Test Method, another *in vitro* test used to assess eye irritation/damage), it can be classified as ocular corrosive or severe irritant without animal tests or further *in vitro* testing. For substances that appeared to be mild to moderate irritants in these *in vitro* tests however, confirmation in the rabbit eye test is still mandatory. For the ICE test, the reason was that in the

retrospective validation study, mild to moderate irritants generally showed the highest inter- and intra-laboratory variability (Prinsen et al., 2017). One might wonder why we use chicken eyes. Is there no material available that is more similar to the human eye? The corneal thickness of pig eyes is more similar to that of the human eye, for instance. The answer is that regulators preferred the use of an alternative test that was similar to the *in vivo* test →



Isolated Chicken Eye test (source: ICCVAM (left image) and Wilson et al., 2015 (right image))

(i.e. the ICE test compares well with the Draize eye irritation test) to an alternative test that was more similar to the human eye (i.e. a test based on the porcine eye would compare better to the human eye; *Prinsen et al., 2017*).

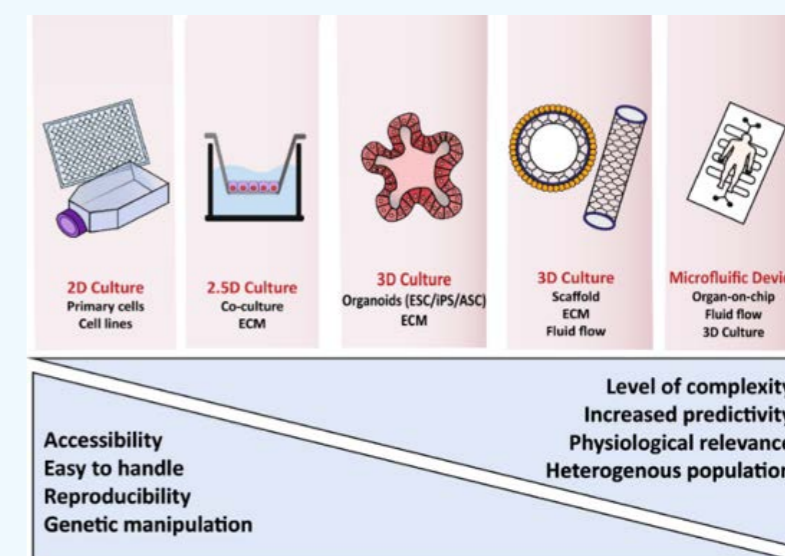
The two endpoints mentioned above (skin corrosion/irritation and eye irritation) both look at basic tissue damage, which more or less can be considered as “cytotoxicity”. However, if one considers skin sensitization things immediately become more difficult. Here, *in vivo* testing is only allowed after *in vitro* tests have been performed; almost 50% of substances tested *in vitro* for skin sensitization will be tested *in vivo* afterwards (Barentsen et al., 2019). When we assess skin sensitization, we look at the elicitation phase of skin allergy which implies that we’re looking at the stimulation of the immune system by a substance. However, this process cannot be captured completely in a single *in vitro* assay because there are too many pathways and cell types involved. In the Adverse Outcome Pathway (AOP) for skin sensitization, four different key events involved are described: 1. Covalent binding of electrophilic substances to skin proteins, 2. Inflammatory/antioxidant response in skin keratinocytes, 3. Dendritic cell activation and 4. T-cell proliferation. Every one of those key events has its own *in vitro* assay, with its own limitations. So how should you validate these types of assays, as not one single assay will replace the *in vivo* situation? We might need a testing strategy combining all these *in vitro* assays; an OECD defined approach for an integrated testing strategy to assess skin sensitization has been drafted (https://www.oecd.org/env/ehs/testing/GL%20DASS_22Sep2019v2.pdf).

The complex issue of complexity

To understand why it is so difficult to validate *in vitro* systems for the replacement of regulatory animal tests, we should first discuss complexity. The endpoints for which validated *in vitro* tests are available involve straightforward processes for which we understand the toxicological mechanism well. For many existing animal testing procedures, this is much more complex. For instance, if we want to fully replace a 90-day toxicity study in rats (e.g. according to OECD TG 413 for inhalation toxicity or TG 408 for oral toxicity), we have to consider the (optional) endpoints investigated in these animal studies such as neuro-behavioural changes, ophthalmoscopy, endocrine effects, bronchoalveolar lavage parameters, clinical observations, functional observations, hematological parameters, clinical biochemistry, body weight changes, food and water consumption, fertility assessment and histopathological assessment of a large amount of tissues. Clearly, it is not realistic to expect that a single *in vitro* assay can generate the same amount of information. If we want to inspire confidence that animal tests, even the more elaborate and complex ones, can be replaced by animal-free alternatives, we will need to introduce more human-relevant complexity into *in vitro* systems.

Of course, we can consider using a battery of *in vitro* tests, so we can test different organ models for instance. A useful approach in this regard is the definition of Adverse Outcome Pathways (AOPs), an example of which was given for skin sensitisation. AOPs describe events that are key drivers in adverse effects, so we know for which

exact event/endpoint we will need to include (or develop) an *in vitro* assay. However, we can only define AOPs for processes we understand very well (a living organism still has many mysteries) and there are limits to the number of *in vitro* assays that are practical to use to replace an animal test. One model for each and every organ or tissue type might be too much to ask.



Different levels of complexity of *in vitro* models – adapted from Faria et al., 2019

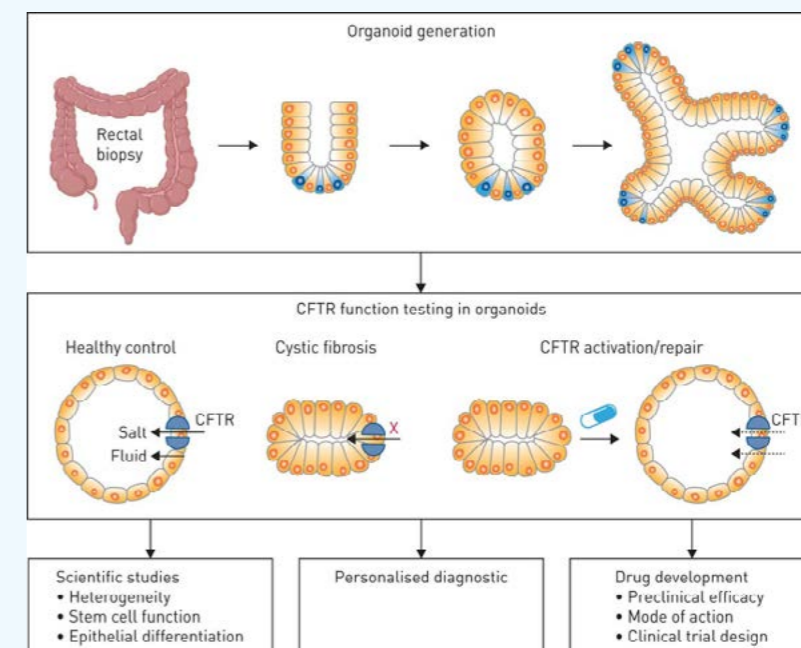
Developing co-culture models is one way to improve *in vitro* complexity, but co-culturing more than a two or three different cell types in one culture vessel quickly becomes very difficult. Another approach is to use tissue material from slaughterhouses (such as bovine or chicken eyes) or, preferably, hospitals (such as human foreskins). This would be expected to be a relatively easy way to improve complexity compared to classic cell culture assays that are based on a single cell line, often of tumor origin. But human material is difficult to obtain and →

setting up an infrastructure for fresh tissues is a major challenge. In the Vital Tissue project (see Cyrille Krul's piece in the current TCDD issue) the possibility of setting up an infrastructure for delivering fresh human vital tissues to researchers is explored. Another initiative to achieve the same main goal (facilitate availability of human tissue to researchers) on a more local level (and with more of a focus on organoid research) is the Utrecht-Platform for Organoid Technology hub (U-PORT) of Utrecht University.

Another issue related to complexity is that *in vitro* exposure is quite different from an *in vivo* exposure; we usually have to dissolve the test substance in an aqueous medium to be able to expose the classically submerged cell culture models. This means that solubility of the test compound can be a major issue. We can mimic a local dermal exposure quite well using for instance a 3D skin model, but oral or inhalatory exposures are much more problematic. A test substance might undergo considerable modification while passing the gastrointestinal tract and is likely to come into contact with several individual organs (e.g. salivary glands, oral mucosa, esophagus, stomach, small intestine, colon); the TNO Intestinal model (TIM) attempts to simulate how the different compartments of the gastrointestinal tract affect the test compound. To assess local toxicity on the different tissue types however, one would need to harvest the modified test substance at the proper phase and use this to expose a well-developed cell culture model of that specific tissue. For inhalation exposure, the physicochemical properties of the substance determine the distribution/accumulation of the test

substance throughout the respiratory tract. Effects in the tracheobronchial region can be quite different from those in the alveolar region, where gas exchange takes place. Vitrocell has developed an *in vitro* inhalation model that has been further developed/characterized at TNO and the RIVM (researchers involved include Ingeborg Kooter from TNO and Flemming Cassee, Yvonne Staal, Hedwig Braakhuis and Evert Duistermaat from the RIVM); with this model users try to "solve" (pun intended) the solubility issue by employing an airway epithelial cell line that is exposed to an air flow including the aerosolized or gaseous test substance on one side and to the nourishing medium on the other side. I.e. dissolving the test substance in medium or in small amounts of solvent is no longer necessary.

The world has seen other major developments that have added complexity and human relevance to *in vitro* systems. The organoid/stem cell field has exploded and tremendous achievements have been made to build a bridge between personalized medicine and pre-clinical testing. Intestinal and kidney organoids (Berkers et al., 2019; Schutgens et al., 2019) from cystic fibrosis patients were shown to be able to predict which treatment option was the best choice for which patient; this is a level of elegance that could never be achieved with animal tests. In fact, this method was successfully tested in clinical trials and has been proven to be so beneficial for patients that health insurance covers the testing procedure. Those who believe that an *in vitro* method can never replace a complex living animal when it comes to predicting effects in humans; you have been proven wrong.



The application of rectal organoids to test cystic fibrosis medication on a single patient level; organoid swelling is observed in healthy controls, but not in CF patients. From Van Mourik et al., 2019

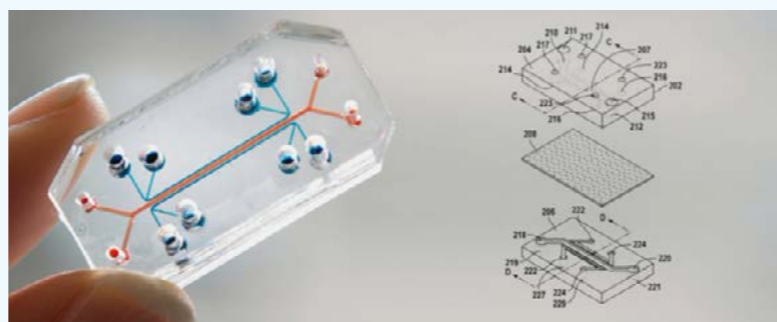
And this is just the start: very recently airway organoids were developed in the same research group that consist of basal cells, functional multi-ciliated cells, mucus-producing secretory cells, and CC10-secreting club cells (Sachs et al., 2019). In the future, we may expect that biobanked stem cells or tumor cells from individual patients allow for testing of patient groups based on their genetic makeup. This will lead to the redundancy of the mouse as the default (and notoriously unpredictable) model organism to study cancer biology and to identify new treatments.

Of course, successful application of an *in vitro* method to study drug efficacy does not mean that such a →

model could be easily implemented for regulatory testing of chemicals/food products. There are still some major hurdles to be taken (or holes in the road to be avoided) before organoids can be used on a large scale in such settings. As mentioned before, availability or tissue/materials can be very problematic (although stem cells and organoid culture protocols can be purchased, for example, at the Hub Foundation in Utrecht). Variability is another issue for organoid culture and we do not yet have proper organoid models for all relevant tissues. Organoids at present mainly consist of epitheloid cells types. This is not yet an organ on a plate or in a well, but a more simple representation of an organ.

To improve differentiation into more different cell types, micro-physiological systems (also known as “organ-on-chip”) have shown to be very useful (e.g. for intestine: Jochems et al., 2019 and for kidney: Faria et al., 2019). Additionally, they can also be used in combination with organoids. Exposing cells to dynamic medium conditions (instead of static conditions) greatly extends the time the culture system can be exposed (28 days exposure is possible in many organ-on-chip systems; even multi-organ-on-chip systems, e.g. Wagner et al., 2013) and shear stress has been shown to stimulate certain cell types to differentiate into more mature cells that are more like the cells in a human organ (e.g. Faria et al., 2019). Also, micro-physiological systems allow for the coupling of different *in vitro* tissue/organ models to investigate their interaction; for instance, a human liver-on-chip could be included to introduce the metabolism component into the *in vitro* system. And a kidney-on-chip with a barrier

system (separating the urine and blood compartments, which necessitates a double flow; such a system is currently in an advanced stage of development in the Roos Masereeuw group) could introduce an excretion component, removing test compound or its metabolites from the medium/blood flow.



Organ-on-chip system from the Wyss Institute/Emulate Inc.

Another possible direction for development of *in vitro* assays is represented by biomarker assays, which look at a specific set of genes to figure out if up/down regulation of these genes causes a specific effect. Examples of these assays are ToxTracker and ReproTracker (from Toxys B.V.) and the GARDassay (from Senzagen).

These are just examples; the intent here is not to provide an exhaustive overview of all possible strategies. It should of course be considered that the more complex an *in vitro* system is, the more difficult validation can be because many factors need to be taken into account. For instance, for micro-physiological systems the choice of materials for the chip system and tubing is very important to avoid compound adherence to the sides/walls, which can greatly affect the concentration the cell system is exposed

to. But the other side of the coin is that if we want to validate an *in vitro* test against human data, a higher degree of (human-relevant) complexity of an *in vitro* system could be expected to be beneficial for the ability to predict effects in humans. This can facilitate validation with the aim of replacing an animal test. Of course, sometimes less is more, and introducing complexity for the sake of complexity (to show off our ability to create complex systems, for instance) should be avoided; it needs to be strictly human-relevant.

NOT EVERY STANDARD THAT GLITTERS IS GOLD

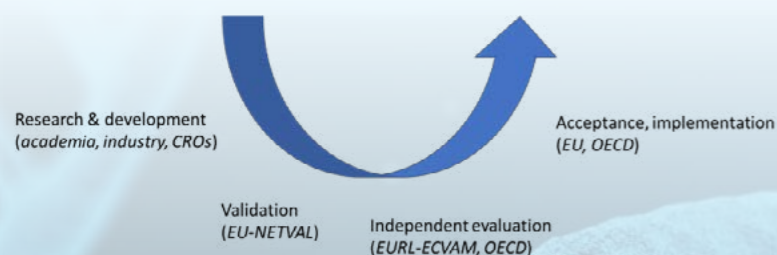
What is the golden standard to validate *in vitro* data against? Ideally, this would of course be human data, but these are scarcely available. Epidemiology studies certainly generate large amounts of data but unfortunately, it is difficult to relate an observed effect to an exposure of a specific substance. So, we often validate against animal data, which are often known to be poorly predictive for effects in humans. For simple endpoints such as irritation, predictivity is quite good but the simpler an endpoint, the larger the potential to develop an *in vitro* alternative... When we consider more complex endpoints, predictivity of animal models is usually not sufficient for accurate risk assessment unless numerous safety factors are included. Unsurprisingly, the addition of safety factors for translating an effect in animals to human risk is common practice. Knowledge on the actual predictivity of animal tests for effects in humans is still often lacking among stakeholders, as these tests have never been validated in the past before they were adopted. →

Predictivity is one thing, but confidence is another major factor determining whether an *in vitro/ex vivo* test will be successfully implemented. The ICE test history (Prinsen et al., 2017) has shown us how difficult this can be, even for a well-validated, reliable *in vitro* test. And certainly, regulators cannot be blamed when they do not accept *in vitro* tests that are poorly characterized/validated; very few of us would be willing to do so. But who are “the regulators”? We’ll get to that in a bit. And to be fair, the situation is quite different now than it was a few decades ago, when the ICE test was considered for regulatory implementation. The societal and governmental pressure to banish animal testing altogether has reached a boiling point, and the kettle is whistling.

VALIDATION OF *IN VITRO* METHODS – HOW IT WORKS

Which stakeholders are involved in the validation process and which regulations and guidance documents are applicable greatly depends on the industry/sector: the situation is very different for chemicals than for pharmaceuticals. For chemicals, the situation is described clearly in the RIVM document “Landschap 3V-methoden risicobeoordeling chemische stoffen” (2017) that can be accessed via the link in the references section.

A simplified visualization of the validation process (based on RIVM, 2017) can be seen in the figure below:



The involved stakeholders:

- EURL-ECVAM (European Reference Laboratory-European Centre for the Validation of Alternative Methods): part of the Joint Research Centre of the European Commission: responsible for drafting the Good *In Vitro* Method Practices guidance document (GIVIMP, OECD, 2018).
- EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods): network of 35 qualified laboratories for validation studies, created in 2013 by the EU. Dutch participants are Charles River Laboratories, (TNO) Triskelion and Wageningen Food Safety Research.

During a validation study, intra- and inter-laboratory reproducibility, relevance of the toxicological effect, the regulatory domain for which the test can be used, advantages and disadvantages, application domains (for assessment of which classes of substances can the test be used) and predictivity (accuracy, sensitivity, specificity) are investigated and determined.

The above describes the validation route for chemicals in Europe: in the US, ICCVAM (The Interagency Coordinating Committee on the Validation of Alternative Methods) evaluates alternative test methods. Also, please note that for *in silico* models, such as (Q)SARs, different criteria are applicable (OECD, 2007).

Similarly, when we consider pharmaceuticals, little of the above applies, although JRC/EURL-ECVAM also work together with the pharmaceutical industry in consortia set up to develop *in vitro* tests. In the process of the development of new medicines, there are two main

phases in which *in vitro* methods might be employed: drug discovery/compound screening and, if we want to replace animal tests, in the pre-clinical phase (pharmacokinetics, safety and efficacy testing). Theoretically, a test method could be readily implementable in drug discovery because no regulations apply. Of course, an *in vitro* method would have to fit the requirements of the pharmaceutical company. Pharmaceutical companies generally perform a high throughput quick and dirty selection process first and then a more thorough GLP selection process of a couple of high potential candidate substances. Usually, they have developed their own screening methods for drug discovery and employ their own validation methodologies. But when we want to replace animal tests by *in vitro* methods, we should rather aim for the pre-clinical phase of the pharmaceutical pipeline. However, there currently is no regulatory framework to validate *in vitro* methods for pre-clinical testing of drug candidates. That doesn’t mean that nothing is being done to replace, reduce and refine animal testing for pharmaceuticals: for instance, the UK National Centre for 3Rs (NC3Rs) and the Dutch Medicines Evaluation Board have focused on reduction and refinement; their efforts and recommendations have led to a changes in ICH and OECD guidelines that led to a reduction of animal use, or even replacement of one of the two species used for certain tests.

THE LONG, WINDING AND BUMPY WAY FORWARD

It is clear that the availability of *in vitro* models that have been validated by an EU-NETVAL-accredited test lab according to OECD and EURL-ECVAM guidelines is presently insufficient. Some of us, including Cyrille Krul (HU), Aldert Piersma (RIVM) and Anne Kienhuis (RIVM), →

are advocating a “revolutionary” instead of an “evolutionary” approach; within the TPI innovation network “Virtual human platform for safety assessment” a conceptual framework for such a virtual human is being constructed (see: <https://www.transitieproefdiervrijinnovatie.nl/english/what-tpi-does-and-supports/virtual-human-for-safety-assessments>). However, such a model would still have to be fed with high quality, reliable data and it is highly probable that at least part of these data is to be generated by well-validated *in vitro* approaches. It is unlikely that for instance the physicochemical properties of a substance of interest would be sufficient for a safety assessment anytime soon.

So how to progress with “evolution”, i.e. validating new *in vitro* methods with the potential to (partly) replace animal tests? Science should certainly keep pushing towards more complex and human-relevant *in vitro* models, as has been mentioned in previous paragraphs. Careful assessment of the reasons for the (s)low availability of validated *in vitro* tests, while stimulating ownership among all involved stakeholders (industry, academia, CROs, regulators), will be key to accelerate the transition. As cliché as it might sound, only by close collaboration can we move forward, at anything more than a snail’s pace.

Some examples of how we might be able to accelerate the transition:

Increased availability of data for validation purposes: a wealth of data on pharmaceutical safety and efficacy has been obtained from pre-clinical and clinical studies. Other companies or product group associations (e.g. Cosmetics

Europe) have also produced large and highly valuable databases that might be used for validation of innovative, animal-free alternatives. Such data are not freely accessible to scientists however, most of the times only their members or regulatory agencies such as CBG-MEB and EMA have (limited) access to these databases. Initiatives such as the Safe Harbor, one of the TPI innovation networks that is coordinated by the RIVM, can greatly facilitate availability of valuable data for *in vitro* validation purposes. In fact, the EMA has created a Safe Harbor for medicines, although the question still is how to provide incentive to companies to use this for data sharing purposes.

Machine learning/data science: machine learning, a type of artificial intelligence, has a lot of potential for much more effective data mining. A prerequisite for successful application is that large, high-quality datasets are available, so that the computer can look for the best combination of *in vitro* assays that might be predictive for toxicity in humans. However, we need to find a way to monitor and check what the computer is doing and how it’s learning. Validation of AI-based methods or moving them into a GLP-compliant environment is at this stage still difficult and the outcome of machine learning platforms always requires expert judgement. The earlier mentioned GARD assay uses machine learning and is currently validated at the OECD, with the aim of developing an OECD test guideline and finally implementing the test. However, the OECD is struggling with the acceptance of this machine learning-based method.

More attention for model development in academia: collaboration between academia and for example CROs and industry needs to improve as well. CROs are not equipped for innovative research and academia is not equipped for method development to a level where it can be used for regulatory testing. The development of new *in vitro* models should be in direct consultation with the companies that represent the end user community. CROs are experiencing more and more difficulties when testing novel substances produced by the industry. This ranges from UVCBs to insoluble substances, mixtures and substances with unknown impurities; these substances have often not been considered when developing and validating the currently accepted tests.

CONCLUSIONS:

The road will be long; acceptance of the ICE test as an alternative to the Draize test in rabbits took decades. Due to the changing political and societal climate, acceptance will be quicker nowadays. But still, it will still take several years or even a decade to fully validate an *in vitro* method for a regulatory testing environment. That is just a single assay, where we might need a test battery of validated tests to replace an animal test. And for many endpoints suitable *in vitro* assays still need to be developed, before we can talk about validation... accelerating the transition is possible, but each km/h has its price tag.

The road will be winding: it’s difficult to see exactly where we will be going on the way to our destination. New scientific developments and breakthroughs will lead us to areas we haven’t foreseen (organoids and →

organ-on-chip exploded onto the *in vitro* field, and artificial intelligence/machine learning is potentially game-changing); sometimes we will have to take a few steps back and rethink our strategies.

The road will be bumpy: as those who have dedicated much of their time to this field know well, this is not an easy ride. We will struggle with harmonization; regulations will have to be adopted on an international level; the Netherlands is forerunner in the field, not every country is ready to accept *in vitro* alternatives to animal tests. We will have to deal with big shots from the old school who have built their careers on animal data and who do not want change, because they believe that might corrode their authority. We will encounter many difficulties in getting exciting, promising developments funded because there is so little money to invest in animal-free progress.

The road will be littered with holes: there are many places this transition can get stuck, at least for some time. It is possible that evolution won't cut it in the end, and we won't get there without a revolution.

But with so many reasons to want this transition to happen (saving costs and time, public opinion/PR and better science/testing), we will certainly reach that destination, sooner or later.

About the authors:

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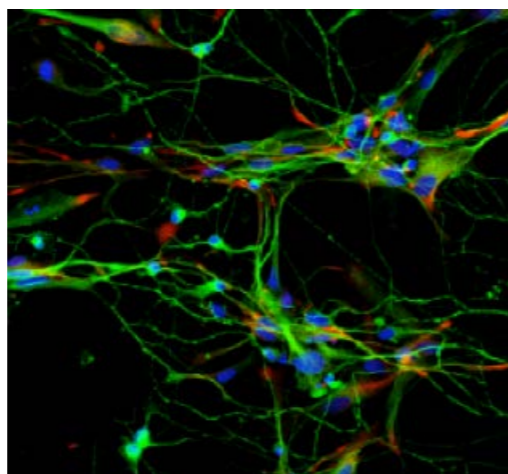
Nomination for the Hugo van Poelgeest Award for excellent animal free research



By: Anke Tukker

Current test methods for neurotoxicity research and seizure liability assessment still rely heavily on large-scale animal, mainly rodent, use. But, as we all know, rodents are not little humans. Thus, the test outcomes are not always predictive for human outcome and moreover, the use of animal experiments is ethically debated. Consequently, this creates an urgent need for more predictive neurotoxicity test methods that preferably use human cells. This circumvents interspecies translation and limits or even eliminates animal use. However, reliable detection of compound-induced neurotoxicity and seizure liability is challenging due to the high degree of integration of neuro-cellular processes. In the project I am working on in the Neurotoxicology Research Group at IRAS (Utrecht University), we try to tackle this challenge and focus on the development and characterization of a human cell-based *in vitro* test model for seizure liability assessment.

At the start of the project, we cultured different human induced pluripotent stem cell (hiPSC)-derived neurons in mono-cultures and showed that the required channels and receptors are present (Tukker et al., 2016). We then created different co-cultures of hiPSC-derived neurons and astrocytes that closely mimic the *in vivo* situation of the human brain.



We showed that these different co-cultures form highly complex networks that become spontaneously active and develop neuronal network bursting in a similar manner as the current gold standard of rodent primary cortical cultures (Tukker et al., 2019). On top of that, these cultures can be modulated with known seizurogenic compounds such as picrotoxin (PTX), 4-aminopyridine (4-AP) and strychnine (Tukker et al., 2019).

Furthermore, we have shown that the ratio astrocytes to neurons as well as the ratio excitatory (glutamatergic) to inhibitory (GABAergic) neurons greatly influences the spontaneous network activity of these cultures and their response to toxicological modulation (Tukker et al., 2018). Our data thus indicate that these models can already be used as a first screen for seizurogenic activity before performing follow-up studies with animals. This research can thus contribute to the reduction of test animals

needed for seizure liability assessment. Therefore, the outcome of this project aids in paving the path towards animal free neurotoxicity testing and seizure liability assessment.

Because my research focusses on finding a test animal free alternative for *in vitro* neurotoxicity testing and seizure liability assessment, I have recently been nominated for the Hugo van Poelgeest

Award. This is the oldest Dutch award for excellent animal free research. Once every four year the Building Blocks for Animal Welfare and the Dutch Society for the Replacement of Animal Testing award this prize. The aim of this award is to motivate young successful researchers working in the field of animal free innovations to continue this work in their future career.

Two other researchers are nominated: Berend van Meer (LUMC) and Carlo Paggi (University of Twente). The next step for all three of us following the nomination is to make a movie about our work. Based on this, a winner will be chosen. This winner of the award will be announced on April 24th, which is World Day for Laboratory Animals. ■

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AIO toxafette - Lennart van Melis

Introduce yourself and tell us something about your PhD project.

My name is Lennart van Melis and this September 2019 I started my PhD project at the Institute for Risk Assessment Sciences at Utrecht University. I did my bachelor's Psychology and Biology and my master's Cognitive Neuroscience at the Radboud University Nijmegen. Therefore, I am both new in Utrecht and in the field of toxicology. My PhD project focusses on the links between endocrine disruption and developmental neurotoxicity at the molecular and cellular level. It is part of a larger collaboration with other European universities in an EU-funded project called ENDpoiNTs. In my project, I investigate the species- and sex-specific effects of exposure to endocrine (ant)agonists and endocrine disrupting chemicals (EDCs) on neuronal function, network



Photo by CDC on Unsplash

formation and maturation. This will be measured using multi-well micro electrode arrays and gene expression analyses. Based on these results, our aim is to develop and validate *in vitro* testing and screening tools, which could be used to identify EDCs that have the potential to induce developmental neurotoxicity.

What are the major challenges that you have encountered in your project?

One of the challenges that I have come across is something I think virtually every PhD student has faced: you have to deal with a lot of uncertainty, which requires you to be flexible. Because I am working a lot with primary cell cultures in my project this is especially true for me, since I never know how my cells will behave or even if I will have any cells to work with in the first place. This means that my planning changes each week, or even during the week itself. It is important to just accept this as part of the PhD experience. Another challenge, which is more specific to myself, is that I did not have a lot of knowledge about toxicology before starting my PhD. In the first weeks and months, many hours have been spent reading and catching up with the developments in this field.

What do/did you expect from your PhD-project?

I started my PhD-project because I really liked to do research and I am always curious about new things. This PhD-project combined my background in neuroscience with something completely new to me, toxicology. I



expect a PhD to be a challenging, but fulfilling and diverse journey, with a lot of responsibility. Besides gaining knowledge, learning new research techniques and writing papers, I believe I will also acquire certain interpersonal 'soft skills' that will help me develop not only as a researcher, but also as a person.

What would you investigate if you have unlimited resources (i.e. time, money, people) in your project?

If I had unlimited resources, I would expand my project in two ways. One approach is more focused on toxicology: currently I am investigating the effect of exposure to specific compounds by directly adding them to →

neuronal cultures. Exposure, however, of course does not work that way. I would also like to investigate what happens to the compounds after they are inhaled, ingested or after skin exposure. Are the compounds transformed and can they pass the blood-brain barrier towards the neurons? And if so, do they still have the same effect on neuronal development? Being a neuroscientist by training, the other approach is more focused on what happens to the brain after exposure. Could the effect of exposure to certain EDCs on neuronal development partly explain some neurodevelopmental disorders and perhaps even lead to finding a cure for these disorders? These are just some of the questions I would like to answer. There are still so many things to discover in this enticing field of neurotoxicology!

How do you combine your PhD-project with your personal life? Are there choices you have to make?

Although it can sometimes be challenging to combine a PhD-project with my personal life, I believe it is important to keep a right balance between the two. I think this can be done by being both flexible and good planning. In my spare

time I like playing football, travelling, being outdoors and having some beers with friends and of course, these things take time. In busy weeks, I am not able to do this as much as I would like, but I try to compensate for that when my schedule allows it. My personal life is very important to me: I would not be able to properly do my work without it!

What are your future career plans?

Since I just started my PhD-research, I don't have a clear view of my future career plans. If I still like doing research in academia after four years, I would gladly apply for an interesting postdoc position. Otherwise, I would be just as happy to pursue my career somewhere else, such as in industry or research institutes.

Are you a member of a society and what do you expect from being a member?

Yes, I recently became a member of NVT. I expect from being a member that I, for example, have access to interesting conferences, have a platform to meet and discuss with other researchers and be able to apply for travel grants. This year I will be part of the committee that

organizes the NVT annual meeting 2020, which in my opinion is a great experience to get to know new people in the field of toxicology and further develop personal soft skills.

Answer to the question of the previous PhD-candidate: Do you consider research communication as an important aspect of your PhD and why so? If yes, to what kind of audience?

Yes, I believe that research communication is a very important in my PhD. For example, I work in collaboration with many other researchers from different universities and it is vital for our research to know each other's results and their implications. Communication, in the form of presentations on congresses, meetings and personal talks, help a great deal to achieve this. Furthermore, I believe it is important to present my own results to larger, non-academic audiences in a clear and concise way. After all, I am doing this PhD project not only for my own development as a researcher, but I am also trying to benefit society as a whole. ■



Source: uu.nl

What is it like to attend the JRC Summer School on “Non-Animal Approaches in Science - Challenges & Future Directions”?

In the period between 21-24 May 2019, the European Commission’s Joint Research Centre (JRC) organized the second Summer School at the JRC in Ispra, Italy. This week-long learning opportunity is dedicated to sharing knowledge about the latest non- animal approaches used in research and testing which include *in vitro* methods as well as computational modelling.

I was given the great opportunity to attend the 2019 version of the JRC summer school together with more than 100 international postgraduate students and young professionals from 34 countries. Next to attending the lectures and interactive sessions including world cafés and participants’ debates, I presented a poster over a part of my PhD research project. During the poster sessions I discussed my research with other researchers and experts from different universities and institutes where valuable knowledge and brilliant ideas were shared. If I’m going to highlight one of

these discussions, I’d no doubt express my gratitude to have had the chance to discuss with Dr. Hanna Karlsson from Karolinska Institute the work that I’ve presented and the possibility to incorporate the techniques used to investigate other endpoints in new studies to be performed in our institutes.

The study I presented was entitled “Impact of *in vitro* digestion on gastrointestinal fate and uptake of silver nanoparticles with different surface modifications”. This study focused on investigating the impact of the biochemical conditions within the human digestive tract on the intestinal fate of silver nanoparticles (AgNPs) across an intestinal *in vitro* model of differentiated Caco-2/HT29-MTX intestinal cells. The grown coculture on permeable membrane was exposed to different concentrations of pristine and *in vitro* digested AgNPs. The AgNO₃ was used as well as ionic control.



By: Ashraf Abdelkhalig
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Toxicology, WUR & WFSR
(previously RIKILT-WUR)

The total Ag and AgNPs size distribution, dissolution, and particle concentration (mass- and number-based) were characterized using ICP-MS and spICP-MS measurements for the apical, cell and basolateral compartments of the intestinal transwell model. We found out that a significant fraction of the AgNPs dissolved during the digestion. Cellular exposure to increasing concentrations of pristine or digested AgNPs resulted in a concentration dependent increase of total Ag and AgNPs content in the cellular fractions. The cellular concentrations were significantly lower following exposure to digested AgNPs compared to the pristine AgNPs.

Transport of silver as either total Ag or AgNPs was limited (< 0.1%) following exposure to pristine and digested AgNPs. We conclude that the surface chemistry of AgNPs and their digestion influence their dissolution properties, uptake/ association with the Caco-2/HT29-MTX monolayer. The outcomes of this study highlight the need to take *in vitro* digestion into account when studying nanoparticle →



toxicokinetics and toxicodynamics in cellular *in vitro* model systems.

At the end of the second day, we had an exciting evening where an interactive quiz challenging our general knowledge took place. On the third day, after the plenary sessions, we visited the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) to see how the work is done in these well-equipped laboratories. The day ended well by having a nice Italian dinner at Villa Quassa with a great view on the Lake Maggiore, the second largest lake in Italy on the southern side of the Alps.

The participants were divided into 8 teams coached by an expert to debate over 4 different toxicological points. Each debate was run by 2 teams where each one must provide arguments and facts that support their side of the debate. I was lucky to be part of an interactive and brilliant team (9 nationalities) to prepare for the “pro” side on a debate over the necessities of legal obligations to support the 3Rs. We worked hard during these preparation days and by the end

of the last day we were able to defend our position and with solid arguments and gained the trust and support of the judges and audience. We argued that legislation can promote quality in research and that by complying with legislation, companies/institutions gain public acceptance and an improved reputation. During the preparation, we had time to learn and know more about different toxicological aspects not only from a scientific perspective but also from the policy maker perspective.

There were several informative and interesting sessions which make it difficult to just highlight a few. What struck me the most was the enormous efforts that have been taken in the EU to support and push forward the 3Rs in science compared to other highly developed countries like Canada. In a presentation over the legislative role in Canada to promote the 3Rs given by Dr. Charu Chandrasekera – the founding executive director of the

Canadian Centre for Alternatives to Animal Methods - I've learnt that 'there is no legislative or policy restrictions that explicitly challenge accepting alternative methods in lieu of conventional studies in Canada'. This reflects the importance of legislation in supporting innovation and the development of new methods and strategies to support the 3Rs.

Overall, it was such an enriching and enjoyable experience. This Summer School gave me the opportunity to explore the efforts and the achievements in the 3Rs domain in science and policy within and outside the EU. ■



International Neurotoxicology Association (INA) meeting in Düsseldorf, Germany

The last conference I attended during my PhD, was the International Neurotoxicology Association meeting in Düsseldorf, Germany. About a year earlier, I sent in a proposal to organize and chair a session and I was pleased to learn that it was granted! The session was designed around the thought that when researching the hazards of psychoactive substances, or substances in general, the exposure should mimic human exposure as well as possible. In this way, data obtained using exposure situations representative of human exposure will be of greater translational value in risk assessment.



By: Anne Zwartsen

The session on possible factors influencing the neurotoxicity of psychoactive substances was scheduled first thing on Tuesday morning. Dr. Michael Baumann from the Designer Drug Research Unit (NIDA, USA) opened the session with his talk on acute *in vivo* and *in vitro* exposure to synthetic cannabinoid receptor agonists newly emerging on the recreational drug market. In his talk he stressed the importance of using concentrations that are relevant for human exposure when determining the hazard. He found that concentrations normally used to test for metabolites *in vitro* are usually several folds higher than in the human situation. As a result, he discovered that different metabolites, and with the wrong ratio, were formed through pathways not used at lower concentrations.

As the second speaker of this session, I wanted to highlight the fact that short exposure durations are mostly used to determine the potency of substances. We determined the potency of new psychoactive substances to affect neuronal

activity of rat cortical cultures following acute (30 min) and prolonged (5 h; average human drug elimination time) exposure. In addition, we determined whether neuronal activity recovered following a 19 h washout period. We showed that prolonging the exposure duration did not exacerbate effects on neuronal activity, suggesting a coping mechanism. Following washout, the recovery of neuronal activity depended on the concentration and drug used. However, when relating the effect concentrations to concentrations relevant for human exposure, several long-acting drugs of interest were highlighted. As these drugs could not have been identified without altering the measurements to better mimic human exposure, we concluded this would be of value to the current hazard characterization of psychoactive substances.

Dr. James O'Callaghan, head of the Health Effects Laboratory Division (CDC, USA), continued the session with his presentation on the influence of stress hormones and →



temperature on the neurotoxicity of amphetamines. He showed that temperature changes induced by the drug solely or in combination with altered ambient temperatures increase neurotoxicity. This can, however, be counteracted by various other drugs like barbiturates and alcohol, and stress due to restraining. However, prolonged exposure to the stress hormone corticosterone contributes to amphetamine induced neurotoxicity.

The fourth speaker of this session was Dr. Giulia Costa from the Department of Biomedical Sciences (ITL). She showed that the strain of mice used in the experiments influences the neuroinflammatory and neurotoxic effects of MDMA. Moreover, the neurotoxicity of MDMA is also dependent on the age at which these mice (adolescents vs adults) are exposed and the gender of the mice. Giulia also determined that caffeinated drinks consumed in combination with MDMA induced neuroinflammatory effects in adolescents, yet not in adults, while at both ages neurotoxic effects were seen.

The session was closed with a presentation of Prof. dr. Ulrike Havemann-Reinecke from the Department of Psychiatry and Psychotherapy (DE). She discussed the current knowledge on the addictiveness of cannabinoids and highlights the fact that a lot is unknown surrounding the effects on the dopaminergic reward system and the glucocorticoid-stress-axis. In addition, she showed that, when acute stress is involved, cannabinoids positively alters complex sensorimotor cognitive functions, which are considered analogue of schizophrenia-like symptoms in humans. Moreover, Ulrike determined that chronic psychosocial stress changes both the balance

of endocannabinoids in the brain and the exogene cannabinoid effects on cannabinoid receptor 1. All in all, the session provided various things to consider while designing experiments for the hazard characterization of psychoactive substances.

Before and after this session, various other sessions were also of great interest. Many presentations were given to highlight the importance of the right *in vitro* model to study the developmental neurotoxicity of substances and various company-supplied cell models were compared to detect the best suitable model to test neurotoxicity. Overall, this

conference was one of the best I went to, and it became even better after receiving the Axion Travel Award for the best oral presentation. I would like to thank the NVT for contributing to my travel and attendance this INA meeting.



Note from the editors:

A travel grant report of the INA meeting, written by Anke Tukker, was included in the previous issue of TCDD.



Designer drugs: Legal, but definitely not safe

Legal highs, designer drugs, research chemicals and bath salts: these are all denominations for a group of close to 900 recreationally used drugs known as new psychoactive substances (NPS). Drugs in this class were first introduced to the drug market in 2004 to induce psychoactive effects comparable to known illicit drugs like MDMA, cocaine and ketamine, while circumventing legislation. The use of these drugs has been linked to many intoxications and even deaths. As common desired and adverse effects are neurological (e.g. euphoria, stimulating, hallucinations, addiction, psychosis, and stroke) it was the aim of my PhD research to determine the neurotoxic effects of NPS.

My PhD research was sponsored by the Dutch Poisons Information Center (DPIC) and was executed at the Neurotoxicology research group at the Institute for Risk Assessment Sciences under the supervision of Dr. Laura Hondebrink, Dr. Remco HS Westerink, Prof. Dr. Dylan W. de Lange and Prof. Dr. Martin van den Berg. Here I aimed at determining the toxicological risks of ~20 NPS and illicit drugs from different categories (amphetamine-type stimulants, cathinones, hallucinogenic phenethylamines, piperazines and arylcyclohexylamines). By reviewing the existing literature on *in vitro* effects at neurological targets we discovered the enormous gaps in the neuropharmacological and toxicological data for most NPS.

We started investigating the effects of NPS on the well-known targets of illicit drugs: the dopamine (DAT), norepinephrine (NET) and serotonin (SERT) reuptake transports. Most NPS and illicit drugs inhibited uptake via DAT and NET potently, while SERT inhibition was

usually less pronounced. As we were able to link potency to several chemical moieties, this would be a first step towards using structure-activity relationships for the estimation of the neurotoxic potential of novel NPS. In addition, the effects of NPS on the different transporters could also be linked to clinical symptoms (e.g. DAT inhibition to stimulants and SERT inhibition to hallucinogenic drugs).

Next, instead of investigating the effects of numerous NPS other potentially interesting neurological targets, we opted for an integrative *in vitro* method to increase throughput. Via the investigation of the effects of NPS on neuronal activity, which we recorded using rat cortical cultures grown on microelectrode arrays, we were able to determine differences in potency to inhibit neuronal activity. The data gathered from these experiments were used to elaborate on the structure-activity relationships made using the transporter data.



By: Anne Zwartsen. Photo: Michel Beakers

Besides characterizing the hazards of NPS, my thesis also focused on improving the predictive value of the available *in vitro* assays by mimicking human exposure more closely. For instance, for the current risk characterisation, short exposure durations are frequently used, while these exposure durations are not representative of human drug exposure. As a result, we determined the effects of prolonged exposure (5 h) and whether neuronal activity was recovered 19 h following the removal of exposure. While prolonged exposure did not exacerbate neurotoxicity, the recovery measurements showed that neuronal activity was not fully recovered for several psychoactive drugs 24h post the start of exposure. What this means for the user remains to be seen, but it shows that investigating the reversibility of effects is needed to identify long-acting substances. →

Another possibility to improve the predictive value of *in vitro* assays lies within exposure temperature. Following drug use, especially following intoxications or overdoses, users can develop hyperthermia. Therefore, we continued improving the predictability of the neuronal activity recordings by determining the effects of drugs under hyperthermic conditions. We determined that the neurotoxic potential of most psychoactive substances increases 1.5-2-fold when measured at 41°C vs. 37°C. This shows the possible underestimation of the hazards of NPS and illicit drugs based on measurements solely done at physiological temperatures.

An additional way to improve hazard characterization is to determine the hazard on a more individual-based level and not for the population as a whole. While interindividual differences are often (partly) explained by polymorphisms in kinetics, the relationship between polymorphisms in dynamics and interindividual variation in drug response is not well investigated and understood. However, our results demonstrate that polymorphisms in the dopamine reuptake transporters can indeed contribute to interindividual differences seen in response to psychoactive substance use. We showed that *in vitro* effect concentrations can vary heavily between the polymorphic and normal dopamine transporter.

We also investigated the effects of NPS on cardiomyocytes as most research focuses solely on the neurological effects induced by NPS and illicit drugs. However, phenethylamines and cathinones are also known to induce cardiovascular symptoms like tachycardia, hypertension and chest pains. While cocaine is known to inhibit potassium,

sodium and calcium channels at low μM concentrations, amphetamine and MDMA are thought to lack these properties and induce tachycardia only indirectly via the increase of norepinephrine. With the use of hiPSC-derived cardiomyocytes, we showed that these drugs and several NPS can directly affect cardiomyocyte functioning. Although from a clinical point of view, tachycardia is unlikely due to direct, acute effects of psychoactive substances during recreational doses, prolongation of the QTc interval can be (partly) due to direct drug effects.

What's next?

For NPS research: there are many ways we could improve the hazard characterization of NPS. For instance, we could

improve the model system used for neuronal activity measurements when hiPSC-derived neurons are well characterized. Moreover, as an NPS is hardly ever taken by itself, co-exposure with other psychoactive drugs, alcohol and medications are of interest.

For me: I successfully defended my thesis on January 31st, 2020 and I just started working as a postdoc in the *In Vitro* Toxicology group at the Institute for Risk Assessment Sciences. Here I am working on including a blood-brain barrier in PBPK models to estimate brain concentrations of therapeutics. ■



Photo: Martijn Bergsma

Upcoming conferences on advanced *in vitro* methods/ alternatives to animal testing 2020

| Event | Time | Location |
|--|--|-----------------------------|
| 2020 RIKEN BDR-CuSTOM Joint Symposium in Kobe – Integrated organoid science: Stem cells, Engineering, Medicine | Wed, 4 March 2020 – Thu, 5 Mar 2020 | Kobe, Japan |
| 59 th Annual Meeting of the Society of Toxicology incl. CAAT Satellite Meeting | Sun, 15 March 2020 – Thu, 19 March 2020 | Anaheim, US |
| Applied <i>In Vitro</i> Toxicology Course 2020 by ESTIV | Sun, 5 April 2020 – Fri, 10 April 2020 | Brussels, Belgium |
| 5th International Conference on Alternatives for Developmental Neurotoxicity (DNT) Testing | Mon, 6 April 2020 – Wed, 8 April 2020 | Konstanz, Germany |
| Dechema 3D Cell Culture conference 2020 | Tue, 12 May – Thu, 14 May 2020 | Freiburg, Germany |
| Workshop on Multi-Cellular Engineered Living Systems (M-CELS) | Tue, 26 May 2020 – Thu, 28 May 2020 | Bethesda, US |
| 12th annual Advances in Cell and Tissue Culture conference 2020 | Tue, 2 June – Wed, 3 June 2020 | Cardiff, UK |
| 7th Annual 3Rs Symposium: Practical Solutions and Success Stories | Thu, 4 June 2020 – Fri, 5 June 2020 | Beltsville, US |
| 21 st international ESTIV Congress | Mon, 8 June 2020 – Thu, 11 June 2020 | Barcelona, Spain |
| Wear It Innovation Summit | Wed, 10 June 2020 – Thu, 11 June 2020 | Berlin, Germany |
| NVT Annual Meeting 2020 | Wed, 10 June 2020 – Thu, 11 June 2020 | Ede, The Netherlands |

| Event | Time | Location |
|--|--|------------------------------------|
| BioCHIP Berlin | Tue, 16 June 2020 – Wed, 17 June 2020 | Berlin, Germany |
| Organ-on-a-Chip, Tissue-on-a-Chip & Organoids Europe 2020 | Tue, 23 June 2020 – Wed, 24 June 2020 | Rotterdam, the Netherlands |
| ISSCR Annual Meeting 2020 | Wed, 24 June 2020 – Sat, 27 June 2020 | Boston, US |
| LIVE 2020: Lung <i>In Vitro</i> event for innovative & predictive models | Thu, 2 July 2020 – Fri, 3 July 2020 | Nice, France |
| EUROoCS Conference 2020 | Wed, 8 July 2020 – Thu, 9 July 2020 | Uppsala, Sweden |
| Summer School Advanced <i>In Vitro</i> Models | Mon, 13 July 2020 – Fri, 17 July 2020 | Utrecht, the Netherlands |
| The 11th World Congress on Alternatives and Animal Use in the Life Sciences | Sun, 23 August 2020 – Thu, 27 August 2020 | Maastricht, the Netherlands |
| EUROTOX Annual Conference 2020 | Sun, 6 September 2020 – Wed, 9 September 2020 | Copenhagen, Denmark |
| Euroensors XXXIV Conference | Sun, 6 September 2020 – Wed, 9 September 2020 | Lecce, Italy |
| IEEE Sensors 2020 | Sun, 25 October 2020 – Wed, 28 October 2020 | Rotterdam, the Netherlands |

*Conferences in the Netherlands shown in **bold**

3Rs student grants 2020: call for submissions



The EPAA has published its call for the 2020 3Rs Student grants. The purpose of the grants is to sponsor students to participate in the major 3Rs events in 2020. They will award one full grant (1000 EUR) and one half grant (500 EUR) for each of the eligible events in: ESTIV2020, the 11th World Congress on Alternatives, and the EUROTOX 2020 Congress.

You can find more information about the grants here: https://ec.europa.eu/growth/content/3rs-student-grants-2020-call-submissions_en



2020 Copenhagen Congress

Message from Prof. Eva Bonefeld-Jorgensen, 2020 Congress President

We look forward to welcoming you to the upcoming 2020 Copenhagen congress scheduled September 6-9, 2020. Online registration and abstract submission for [EUROTOX 2020](#) is now open.

“Toxicology of the next generation – Combined efforts in quest of safer chemicals and medicines” is the theme of the EUROTOX 2020, and promises a diverse [congress programme](#) including a variety of topics dealing with safety of drugs and environmental chemicals, new and emerging technologies, personalised medicine, human health effects caused by exposure to chemicals as well as safety issues arising from climate changes.

Join us for four eventful days in the Tivoli Congress Center, located in the heart of Denmark’s beautiful capital. Don’t miss more than 30 top-notch scientific sessions, keynote lectures from renowned scientists, and outstanding Continuing Education Courses (CECs), along with networking opportunities and social activities.

IMPORTANT DEADLINES

- Abstract Submission and Travel Bursary Application: March 31, 2020
- Registration at the Early Bird Fee: May 14, 2020

Stay tuned for congress highlights.



Life2020: call for abstracts



THEME: NETWORKS IN LIFE

WHAT IS LIFE | Life is a unique, inspiring, annual national congress covering all disciplines and scales in the Dutch Life Sciences, from femtometer to kilometer, from cell to planet. Life2020 will be the second edition. The first edition, Life2019, was a success. To get an impression view the aftermovie at www.nwolife.nl.

WHY LIFE | To connect senior and junior researchers from all different disciplines in the life sciences. To explore and push boundaries. To discuss new or desirable developments in the field. To get inspired by each other's research and approaches and to start new interdisciplinary collaborations.

The theme for this second edition of Life is 'Networks in Life'. Networking and interdisciplinary collaboration is essential for researchers in the Life Sciences. Networks are also essential in life at all scales, from cellular, fungal and neural networks to ecological networks.

WHO ARE INVITED | Researchers at all scientific research institutes including the university medical centres in The Netherlands, in all career phases including master students are warmly invited to join and contribute to the programme! People working in the industry, policy makers and Readers and their master students at Universities of Applied Sciences (HBO's) are particularly invited to join.

DATA | 26 and 27 May 2020

VENUE | Hotel Zuiderduin, Egmond aan Zee
<https://zuiderduin.nl/zakelijk>, near the beach.

FORMAT | A plenary programme with keynote speakers and 5 parallel tracks for oral presentations and discussions as well as interactive plenary sessions, two poster sessions, an Innovation Floor, workshops, master classes, focus sessions and a social programme. The 5 parallel tracks, covering both congress days, address the theme 'Networks in Life' at different scales.

CALL FOR ABSTRACTS | Participants are invited to submit abstracts for oral presentations (max. 15 minutes) or poster presentations. Three poster prizes are available for junior poster presenters. While submitting, be aware of the broad audience.

Tracks (longer descriptions on the website):

1. Biomolecular networks within and between cells | This track will focus on molecular networks within and between cells that underlie central processes such as metabolism and development in all domains of life. Knowledge and control of these processes is not only important for industrial exploitation of organisms and in the fight against devastating diseases, but fundamental to our understanding of the diversity, flexibility and robustness of life. [Read more...](#)
2. Genome regulation: how tissues and organisms form and survive | This track is about the regulation of our genome and its effect on an organism. It covers the

broad fields of (epi)genetics and genome biology in the context of development, (patho)physiology and evolution of tissues and organisms. [Read more...](#)

3. Interactions with the environment: for better and for worse | This track is about how organisms interact with their environment at the local (e.g. family, territory) level, and how this leads to beneficial or detrimental outcomes for the organisms themselves and/or the environment. This has both fundamental and societal relevance. [Read more...](#)
4. Life and Planet | Networks in Life on our Planet are fundamental for maintaining biodiversity and ecosystem functioning. Within the track 'Life and Planet' we aim to highlight the importance of these interactions, as well as the processes that affect the structure and stability of the networks on a species level and above. [Read more...](#)
5. Connecting the dots: novel approaches in biological measurement and analysis | This track focuses on new methods and technologies for measurement and analysis that power today's scientific discoveries. We invite contributions on biological systems of all scales and all levels of organisation. Contributions go beyond pure technical detail and provide insight in the trends in the particular domain of research. [Read more...](#)

All tracks will contain a mixture of invited speakers, and speakers selected from the abstracts. The 5 tracks will cover both days of the congress.

Deadline for abstract submission for oral presentations is March 1, 2019 Deadline for poster abstract submission is April 1, 2019.

[Submit abstract](#) ■

Christmas puzzle solution and winner

Peter van Kessel wins again by submitting the solution to last year's Christmas Puzzle: "**Merry Christmas from the TCDD editorial team**".

Congratulations! Chance is really on your side as we select winners using a random draw process. For those who have been sending in the correct results to the puzzle over the years and did not yet win, do not give up. Next time it might be your chance.



An EST christmas tree!

Photo competition Winner!

This is the first year that we launch this competition and to remind you, we asked an office/lab to construct (or if you prefer misconstruct) a Christmas tree and send us the picture. The winners were undoubtedly **Gina Mennen en Victoria de Leeuw from the RIVM**.

EPA NEWS

Updated List of Alternative Test Methods and Strategies/ New Approach Methodologies (NAMs)

EPA released an updated version of its List of Alternative Test Methods and Strategies (or New Approach Methodologies [NAMs]). The document can be viewed [here](#). The list contains links to test guidelines, method protocols, computational tools and databases, and guidances and policies from EPA that "do not use vertebrate animals to develop new data/information," and additional guidance or other documents that are considered "tools and approaches which may enhance the use of NAMs for regulatory use under TSCA."



Renewed and new courses within the Postgraduate Education in Toxicology Programme



The Postgraduate Education in Toxicology (PET) offers 19 courses that are since 2018 officially recognized by the EUROTOX. With this achievement, PET is the first fully recognized European training programme for toxicologists. To maintain the high quality of the courses, which is also required for EUROTOX recognition, course coordinators and lecturers continuously update their course material. However, to keep on providing the best toxicological education programme in Europe, it is also important to invest in renewing existing courses and to broaden the programme with new courses which fill-in gaps within the toxicological field that are currently not part of the programme. With this in mind, a total of 3 new PET courses (Neurotoxicology, Exposure Assessment and Current Topics in Toxicology) have or are being developed and 4 courses (Pathobiology and Toxicological Pathology, Epidemiology for Toxicologist, Legal and Regulatory Toxicology and Risk Communication and Perception) have or are being renewed by the course coordinators taking into account evaluations of participants as well as input from the PET supervisory board and the Concilium Toxicologicum. Below you can find more details on the course, including their new or renewed content and the date of their first edition. Please check the www.toxcourses.nl website for more information on the full PET programme.

IN 2019

NEW COURSE: NEUROTOXICOLOGY

Coordinator: Dr. Remco Westerink and Drs. Anke Tukker
Location: Distant learning and on-site classes at IRAS - Utrecht University
Next course: 8 – 14 April 2021

The course Neurotoxicology (held for the first time in 2019) is the first course in Europe focussing specifically on neurotoxicology. This course makes use of two days of distant learning with e-lectures and webinars by international experts and three days on-site. During the course, the participants will acquire a strong understanding on the principles of neurotoxicology, the mode of action of major neurotoxicants, the state of the art of neurotoxicity testing and how to assess the neurotoxic hazard and risk of exposure to specific neurotoxicants.

RENEWED COURSE: PATHOBIOLOGY AND TOXICOLOGICAL PATHOLOGY

Coordinator: Dr. Aswin Menke
Location: Dept. Pathology - Utrecht University
Next course: 5 – 9 October 2020

The renewed course on Pathobiology and Toxicological Pathology has a stronger focus on toxicological pathology. Furthermore, the anatomy and histology is more directed towards the animal species that are generally used in toxicological research. During the 5-days on-location, the participants will acquire a basic understanding of general and toxicological pathology and will have sufficient knowledge to understand and interpret pathological data in papers and reports.

| PET course | 2020 | 2021 |
|------------------------------------|----------------|--------------|
| General Toxicology (M) | Continuously | Continuously |
| Molecular Toxicology (M) | 29 Jun - 3 Jul | July |
| Cellular Toxicology (M) | 14 - 17 April | April |
| Organ Toxicology (M) | - | 11 - 15 Jan |
| Epidemiology for Toxicologists (M) | 11 - 15 May | - |
| Ecotoxicology (M) | - | 18 - 29 Jan |
| Pathobiology (M) | 5 - 9 Oct | October |
| Laboratory Animal Science (M) | - | November |
| Risk Assessment (M) | 12 - 16 Oct | October |
| Toxicogenomics (O) | - | 1 - 5 March |
| Food Toxicology (O) | - | 23 - 27 Aug |
| Occupational Toxicology (O) | 8 - 12 June | - |
| Immuno Toxicology (O) | 23 - 26 June | - |
| Reproductive toxicology (O) | 26 - 30 Oct | - |
| Legal & Regul. Toxicology (O) | 23 - 27 Nov | - |
| Med. & For. Toxicology (O) | 2 - 10 Nov | November |
| Carcinogen. & Mutagenesis (O) | - | 8 - 12 Feb |
| Risk Communication (O) | - | 17 - 21 May |
| Neurotoxicology (O) | - | 8 - 14 April |
| Exposure Assessment (O) | - | May / June |

(M) = Mandatory
(O) = Optional

IN 2020**RENEWED COURSE:****EPIDEMIOLOGY FOR TOXICOLOGISTS**

When: 11 – 15 May 2020
Coordinator: Prof.dr. Majorie van Duursen
Location: VU University of Amsterdam

The renewed course on epidemiology is specifically developed for toxicologists. During the 5-days on-location course, participants get familiar with the principles, basic terms and working areas of epidemiological research. Participants learn how epidemiological findings can contribute to the knowledge within the field of toxicology. The strengths and weaknesses of epidemiological study designs (observational and experimental) will be discussed. In addition, the participants learn about measures of frequency and association, and sources of bias. At the end of the week, participants are able to interpret epidemiological findings and write a research proposal for an epidemiological study within the field of toxicology.

RENEWED COURSE:**LEGAL AND REGULATORY TOXICOLOGY**

When: 23 – 27 November 2020
Coordinator: Ms.ir. Astrid Bulder
Location: Distant learning and on-site at RIVM, Bilthoven

In order to address in depth the different topics of Legal and Regulatory Toxicology this renewed course has been extended from 3 to 5 days. The course makes use of one day distant learning with e-lectures that provide the participants with general knowledge on Legal and Regulatory Toxicology. On-site, the course will go in more depth into the different legal and regulatory frameworks that exist in the risk management of chemical substances. Overarching aspects such as animal testing and good laboratory and good clinical practices will also be discussed. By working on case studies, the participants will acquire a strong understanding of the role(s) of toxicologists in the field of regulatory toxicology.



Photo by ThisisEngineering RAEng on Unsplash

IN 2021**NEW COURSE:****EXPOSURE ASSESSMENT**

When: May / June 2021
Coordinator: Dr.ir. Paul Scheepers
Location: Radboudumc, Nijmegen

The new course Exposure Assessment is developed to tackle exclusively the exposure assessment within the risk assessment in toxicology, which is often seen as the most challenging step in the risk assessment process. During the 5-days on location, the participants will acquire a thorough understanding on the principles of the exposure science in the field of toxicology, taking into account different exposure and absorption routes of chemicals. Strengths and limitations of quantitative exposure assessment methodology will also be discussed. Ultimately, the participants will be able to use exposure data and models in the risk assessment of chemicals.

RENEWED COURSE:**RISK COMMUNICATION AND PERCEPTION**

When: 17 – 21 May 2021
Coordinator: Dr. Marianne Bol-Schoenmakers and dr. Mieke Lumens
Location: Distant learning and on-site at IRAS – Utrecht University

The course Risk Communication and Perception is being renewed to meet the need for training in risk communication and perception for toxicologists, to enhance public understanding in risk assessment. This need had been expressed recurrently by toxicologist with different backgrounds. The course will make use of distant learning (e-lectures, webinars and online assignments) combined with days on-site. During the course the participant will 1) gain knowledge on several ways by which experts, civilians, police makers, media and interest groups approach risks; 2) choose for a more informative or more persuasive communication strategy, based on arguments; and 3) understand and facilitate risk communication processes approach risks.

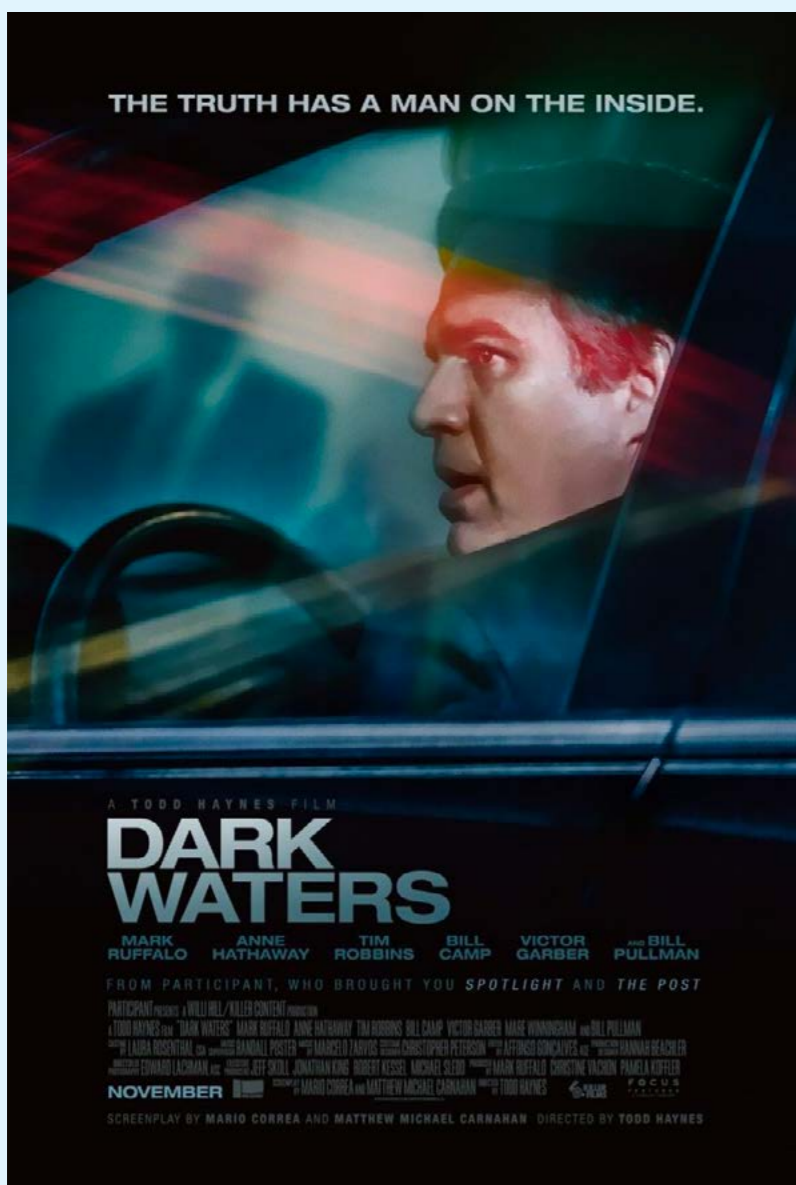
NEW COURSE:**CURRENT TOPICS IN TOXICOLOGY**

Expected: April or September 2021
Coordinator: Prof.dr.ir. Juliette Legler
Location: Utrecht

This new course is intended for (registered) toxicologists who would like to keep up to date on the latest developments in the field of toxicology. The course will consist of 3 parts, namely an afternoon, evening and subsequent morning session in which new insights and perspectives in key current topics in toxicology will be presented.

Toxicologie in de media

Dark Waters



Zoals wij als toxicologen weten is Toxicologie overal om ons heen, zo ook in de verschillende media. In "Toxicologie in de Media" willen wij aandacht besteden aan opvallend (in de breedste zin) toxicologie-gerelateerd onderwerpen dat recentelijk in de media geweest is, serieus of populair.

In deze editie daarom aandacht voor de film "Dark Waters", die eind vorig jaar in première ging. De film is gebaseerd op de lange, juridische strijd die een advocaat eind jaren 90 voerde tegen chemiebedrijf DuPont, producent van Teflon, uit Parkersburg (West Virginia, USA). De advocaat was ingeschakeld door een lokale veehouder die claimde dat DuPont plaatselijke wateren had vervuild en daarmee zijn vee ziek had gemaakt. Tijdens de juridische strijd tegen het bedrijf bracht de advocaat een decennialange geschiedenis van milieuverontreiniging aan het licht, met name de negatieve effecten van perfluorocanzuur en PFAS.

Interessante toevoeging: Eerder dit jaar, op 5 Februari, bezocht producent en hoofdrolspeler Mark Ruffalo de Europese hoofdstad Brussel, om de film te presenteren aan de president en leden van het Europees parlement.

Mocht u dus een avondje niks te doen hebben... Wat ontspanning met een knipoog naar de toxicologie!

Toch liever een wat meer wetenschappelijke kijk op het onderwerp? Bezoek dan 7 april aanstaande het symposium van de NVT sectie Risk Assessment over dit onderwerp. Meer details over dit symposium en de agenda kun u vinden elders in deze TCDD onder "Nieuws van de Secties".

Voor meer informatie over PFAS vanuit een EU perspectief, bezoek <https://www.eea.europa.eu/themes/human/chemicals/emerging-chemical-risks-in-europe> en voor specifiek Nederlandse informatie, bezoek: <https://www.rivm.nl/pfas>.

Bamboo cups: dangerous and not green

The German Stiftung Warentest discovered that so-called reusable bamboo coffee cups with hot drinks such as coffee release the harmful substances melamine and formaldehyde. The research can be read [here](#) (in German).

By Daniël Linzel, subject and web editor at C2W (member magazine of the KNCV)

Bamboo coffee cups are often offered as a sustainable alternative to disposable cups. They are said to be biodegradable and therefore not harmful to the environment. Stiftung Warentest examined these claims and reported on them on their website.

The cups are not made purely from bamboo, because it also contains plastic that consists of the components [melamine](#) and [formaldehyde](#). In the plastic, the components are stuck together and under normal circumstances there is no danger, as long as the temperature remains below 70° C. And there is the problem.

The researchers poured a slightly acidic, hot 70 ° C liquid into twelve different cups and let it sit at that temperature for two hours. They repeated this test seven times and after the third and seventh time they viewed the content. The results showed that considerable amounts of melamine were released in seven of the twelve cases, as well as formaldehyde. Melamine can affect your kidneys and bladder, and formaldehyde can cause irritation and in

serious cases cause cancer in the nose and throat upon inhalation.

Another problem with the cups is that they are often not properly labeled. Because the cups consist of both bamboo and plastic, they are not biodegradable and composting therefore does not work with this material. But the labels usually do not state that it contains melamine and refer (vaguely) to the biodegradability of the cup. The advice of Stiftung Warentest: stay away from these types of cups.

[Here](#) you can find details about the fabrics and labeling of the twelve brands.

This article can be found here: <https://www.c2w.nl/nieuws/bamboebekers-gevaarlijk-en-niet-groen>

The opinion of the Bundesinstitut für Risikobewertung (BfR) can be found here: <https://www.bfr.bund.de/cm/343/gefaesse-aus-melamin-formaldehyd-harz.pdf>



Bron: <https://www.c2w.nl/nieuws/bamboebekers-gevaarlijk-en-niet-groen>



Photo by Mike Marquez on Unsplash

TCDD is de nieuwsbrief van de Nederlandse Vereniging voor Toxicologie (NVT).

De Vereniging beoogt de belangen van het vakgebied Toxicologie in de ruimste zin te behartigen; de Vereniging heeft uitdrukkelijk niet de bedoeling de rechts-positionele belangen te behartigen van de individuele leden, tenzij deze belangen direct gerelateerd zijn aan de beoefening van het vakgebied. Gehele of gedeeltelijke overname van de inhoud van TCDD is alleen mogelijk met schriftelijke toestemming van de redactie.