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SPECIAL THEME

PFAS Issue Persisting

- PFAS Alternatives and Degradation Technologies
- Health Effects of Pfas – Do We Even Know?
- The State of Affairs of PFAS Regulation
- Proposal for an EU/EEA-wide Restriction on PFAS

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Inhoud

Future-proofing Working Group - **Invitation to Participate!**

To future-proof our society and make sure we continue to connect with our members' interests and developments in the field, the NVT board sent out a questionnaire last year. The aim of this questionnaire was to understand the opinion of the NVT members on topics like how current sections fit with the field, the future of the NVT sections, topics to be added and how to promote interactions and collaborations between sections and other societies.

The questionnaire was completed by 64 respondents in total, of which 83% were also members of (one of) the NVT sections. Respondents clearly indicated a need for more interactive meetings, better communication and promotion of current activities, and a wish to further collaborate with other societies. Topics that should be addressed by the NVT or its sections or need to get more attention in general were: NAMs, clinical toxicology, computational toxicology and regulatory toxicology. There were also differences in some of the responses: around 20 respondents voted for more focus on research in toxicology, an equal number of respondents voted for more focus applied toxicology. Also, there was equal preference for 'continuing the current sections' and 'restructuring of sections to current needs and developments'. Food for further discussions!

In parallel to the questionnaire, the section boards discussed the future of the sections, in early February, initiated by the section Risk Assessment. During the round table meeting the sections addressed additional considerations for future-proofing NVT sections, including moving organisational tasks of the section meetings to the NVT secretariat, professionalising communication, and spreading the events over the year. As some sections see a reduction in memberships and decreased participation in their activities, an increasing need for collaboration is identified as well as a re-evaluation of the identity of the sections and their topics. Organizing special interest groups or parallel sessions during (larger) activities such as the NVT Annual meeting was suggested as a way forward. Overall, the results of this exchange align quite well with the outcome of the questionnaire.

You'll hear more details about the outcome of the questionnaire and the plans for further future-proofing during the NVT Annual meeting on June 8th during the business meeting.

The outcome of the questionnaire, as well as the exchange of the sections, point towards a need for further discussion on how to future-proof the NVT and its sections. To this end, the NVT board proposes to start a working group 'Future-Proofing' to further discuss and implement next steps with representatives from the board, sections and members. Themes that would need to be further discussed are:

- Professionalisation of NVT communication
- Increased role the NVT secretariat in the organisation of section meetings
- Organization of combined meetings by several sections
- Combination of sections?
- ....other??

The NVT board is inviting you as representative from either a section and/or as a member to participate in this working group and help us in Future-Proofing the NVT on one of these themes.

Please contact us in case you are interested to join, at secretaris@toxicologie.nl

On behalf of the board,

Anne Kienhuis
and Joanne Salverda

TERUG NAAR INHOUD

Editorial

At the beginning of the year, I joined the editorial team of the TCDD. Therefore, I would like to start by introducing myself. I am Marcha Verheijen, 36 years old, and I have been working at the department of Toxicogenomics at Maastricht University since April 2014. I started out as a PhD-student focused on transcriptomics changes in 3D cardiac microtissues after treatment with chemotherapeutic drugs. On May 17th, 2019 I defended my thesis entitled “Transcriptomics close to my heart: Advanced models & methods for Toxicogenomics research”. But my journey at the department was not over yet. I continued working there as a postdoctoral researcher where I first spent my time developing the R-ODAF, a transcriptomics preprocessing pipeline for regulatory use. Thereafter, I continued with researching microRNAs in Alzheimer’s disease. Currently, I am working as a data manager for the ONTOX project and spending my time to bring my transcriptomics analysis knowledge to the next level, focusing on single cell technologies and other cool ways to get more information from omics data. In the future, I hope to expand my knowledge to the field of epigenomics to obtain a better view of regulatory processes that change in a cell after exposure to a toxin.

In this TCDD, we revisited the PFAS Issue to see if it is Persisting. PFAS (Per- and Polyfluoroalkyl Substances) are all around us. They are used in so many products for their water-resistant, non-stick and/or fireproofing properties. But they have also been accumulating in the environment and our own bodies because they are don’t break down easily. In this issue we included, amongst others, articles related to health effects (page 11), regulation (page 13), actions to ban PFAS (page 16) and a funny reflection of what the world would look like if all products containing PFAS would magically disappear tomorrow (page 23).

Next to PFAS, this issue also includes the vision of Thomas Hartung on the future ToxAIcology (page 18) and how a chemical spill can become a major disaster if not acted appropriately (page 20).

Hopefully, you do not have too much PFAS in your body, so that the information will stick ☺

On behalf of the editorial team,

Marcha Verheijen

Photo by Jess Bailey on Unsplash

SECTION RISK ASSESSMENT

Report Online Spring Symposium April 4th 2023
Green Toxicology: How big is our footprint?

Welcome & Introduction
The chair welcomes everyone to this meeting of the risk assessment section of the NVT.

Before giving the floor to the speakers, a few questions on the expectations of this meeting and the experience with sustainability in the lab are answered by the audience. There is a general interest in options to improve our footprint, what is going on in this field, what are the quick wins. Most attendants have limited personal experience in this field, but are curious to hear what is going on.

LEAF – Addressing the Sustainability of Science, by Martin Farley (UCL)
LEAF (Laboratory Efficiency Assessment Framework) is a standard to improve sustainability of Laboratories. Many institutions and companies have net-zero targets, but what does that mean for our scientific laboratories? Laboratory facilities consume lots of energy, think of ventilation, heating, cooling, fume hoods, freezers, ovens, and autoclaves. It is also a sector that is growing. Companies might reach net zero for scope 1 (direct emissions) or scope 2 (indirect emissions), but in most cases not for scope 3 (e.g. purchased goods). The largest impact on sustainability is in fact related to what we buy, which makes it very complex as new products are often more energy efficient in use, but come out worse in a life-cycle analysis. Also keep in mind that producers try to push us to buy more. On the positive side, there are also producers that are running net zero on energy use. However, many companies have not done a real life-cycle analysis, which is the golden standard for measuring impact. Always aim for absolute figures on carbon emissions. In general, washing and reusing saves carbon over using single-use materials. What is also needed is more scientific research on how to achieve net-zero science, as well as standards for sustainable practices, of which LEAF is an example, which includes a calculator of carbon and money saved. LEAF has been online for 2 years and has achieved a large user base in that time. Further in formation and materials can be found at https://www.ucl.ac.uk/sustainable/leaf-laboratory-efficiency-assessment-framework

Sustainability at the RIVM, by Eline Politiek (RIVM)
Eline is the sustainability coordinator at RIVM. RIVM has challenges with regard to sustainability due to the spread over multiple, mostly older, buildings. This makes RIVM a relatively high contributor to governmental CO_2 use. The goal is to reach the government goal of climate neutral business operation in 2030. To this end the RIVM has been using the CO_2 performance ladder (prestatieladder) with certifications at several levels, since 2022. The first action was to purchase green electricity. Next steps are ventilation, better isolation of buildings, more led lights. But the biggest impact is in purchase; this will be addressed with MVOI (Maatschappelijk Verantwoord Opdrachtgeven en Inkopen), but it is still a challenge to incorporate sustainability in purchase orders.

For personal transportation, the bicycle is encouraged financially. Waste is an issue as the waste company does not accept the separated streams. Success was achieved with the harmonization of lab supplies between the seven departments. The first project was on the use of pipettes, now everyone uses the same type of pipette tips, with more efficient ordering and discussions with suppliers about the packaging. The second project was on gloves, a reduction from more than 20 types and brands to only 2 types was achieved.

The problem is that sustainability is important but not urgent, and quality and safety come first. A complication lies in the plans of the RIVM to move to the new building in 2025, which should have taken place in 2018. And the bureaucracy is very time consuming. For the future the performance ladder (prestatieladder) helps a lot as it measures progress and raises awareness, as well as being part of LEAF. Lastly, working together between institutes gives us more strength. ⇨

Balancing best practices, regulatory compliance, and sustainability in a toxicology lab environment, by Liesbeth Segers (Charles River Laboratories)
Liesbeth is Environmental Health, Safety and Sustainability manager at Charles River, a US Contract Research Organization. Sustainability has been on the agenda for the last 25 years. The facility at Den Bosch offers safety assessment for new and existing substances like pharmaceuticals and chemicals. Also, there are facilities in Schaijk, Leiden and Groningen. They work according to Good Laboratory Practice (GLP), have animal welfare accreditation and the Ecovadis label, which is a very broad sustainability certification. As they test new substances, the safety characteristics of these substances are often not known yet. The first priority is safety, followed by quality, efficiency and sustainability. There are two incentives: specific budget for sustainability measures and cost saving due to energy reduction. In addition, sustainability is made a goal in every employee's and team's performance.

Some examples of actual measures to improve sustainability were given: 1. Change the anesthetic gas from isoflurane to CO_2 to have less emission and replacement of F and Cl but to consider challenges like animal welfare, safety (no smell) and quality. 2. Recycling or replacing formalin for the fixation tissues, to increase safety (CMR decrease) and to reduce emission and less energy on waste destruction, but to consider e.g. more handlings in case of recycling and quality issues. 3. Decrease over-ventilation by using smaller fume hoods and also using less chemicals so that you need less ventilation. The challenges are the requirements for animal housing and worker safety, high investments for air handling, but the win is to have less energy use due to a decrease in heating and cooling. The goal is to get the building from the seventies to net zero. In the US and Spain, they have their own windmill parks. 4. Packaging is also a challenge. Some jerry-cans can be sent back to the suppliers, but they cannot be reused as long as they are not from the same plastics. An important factor is to involve all relevant teams and colleagues as well as setting up project teams for changes, to share knowledge, talk with suppliers and contractors and focus on chemicals, materials and energy.

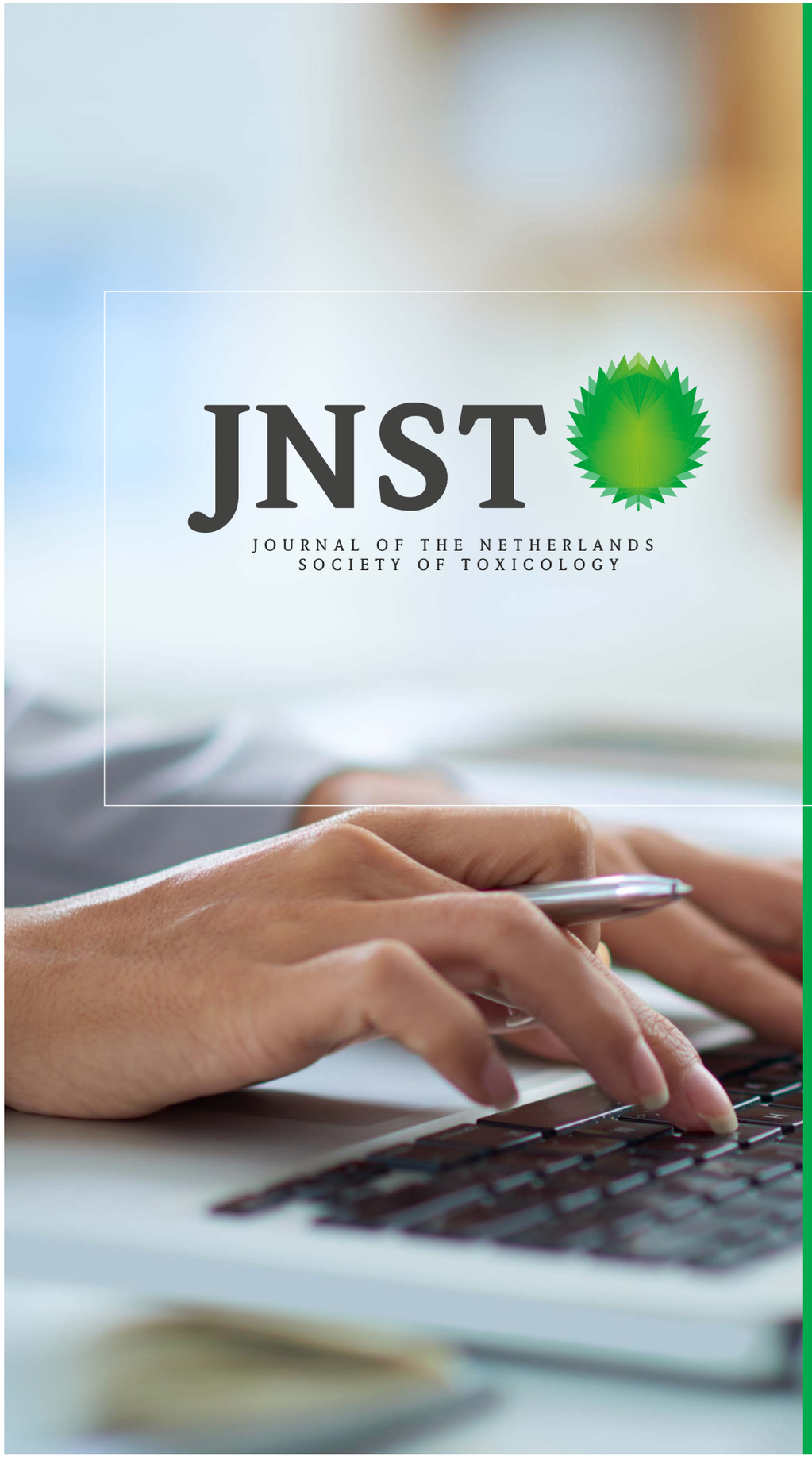
Discussion & wrap-up
In general, the feedback is very positive. After each presentation the audience and speakers use the opportunity for interaction and discussion. The scope of sustainability appears to be wide and there is a need to balance multiple aspects (e.g. sustainability versus safety). There are many useful improvement opportunities in labs and buildings, and it was stimulating to discuss them with each other.

In the end we can best focus on the part we can do ourselves. Our own slice of the apple pie as it were.

Hopefully this helps to start more actions and gets people to take action.

Even little steps can help a lot and can make a difference.

The presentations can be found here.



JNST

JOURNAL OF THE NETHERLANDS SOCIETY OF TOXICOLOGY

We are delighted to announce the launch of a new journal!

Call for submissions

to the Journal of the Netherlands Society of Toxicology

- Submissions can be made through ScienceOpen starting the 15th of June 2023. A "submit a manuscript" button will be available at that point in time.
- A free account must be made with ScienceOpen prior to submission.
- Author guidelines can be found by following this link: Journal of the Netherlands Society of Toxicology – ScienceOpen.
- There is no deadline for submission.
- Once the submitted papers are accepted and have completed the peer review process, they will be available online and the journal entries will be appended to the TCDD.

PFAS Alternatives and Degradation Technologies

Ever-present, PFAS not only needs to be replaced but also degraded

By *Barae Jomaa*

Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals that, together with plastics, have enabled our modern lifestyle. Little did we know that PFAS would persist in the environment and accumulate in our bodies. In fact, according to data from the National Health and Nutrition Examination Survey, PFAS can be detected in the blood of 98 percent of US adults. That is a concerning figure, especially when we consider that PFAS have been linked to adverse health effects, including cancer, reproductive and developmental toxicity, as well as immune system toxicity. It is no wonder then that governmental agencies across the globe have been hot on the heels of the chemical industry, urging the replacement of PFAS and, in some cases, seeking to ban certain uses altogether[1]. As PFAS use gets restricted, it is important to understand what alternatives are available and whether it will be possible to develop technologies that will be able to clean up our environment.

While the use of PFAS is widespread (figure 1), this article will focus on a selection of use categories and explore alternatives. Later on, PFAS degradation technologies will be highlighted so that we can envisage a future where we have overcome our exposure to this class of chemicals.

Figure 1: Sources of PFAS. Image credit and copyright: Commonwealth of Australia, Department of Defence. https://defence.gov.au/environment/pfas/pfas.asp

PFAS have been used in a wide range of consumer applications due to their unique properties, including water and oil repellency, heat resistance, and non-stickiness. Some of the consumer uses of PFAS include non-stick cookware, food packaging, textiles, personal care products and electronics.

I - USES AND ALTERNATIVES

1) Non-stick cookware and bakeware

As they are resistant to heat, water, and oil, PFAS have been used to coat non-stick cookware. For some years now, PFAS-free cookware has been on the shelves. The most popular solution has been ceramic-based coating. These provide a similar function with the added benefit that ceramic coatings do not peel off into the food that is being prepared. Silicone is yet another alternative. It can be used to coat cookware but also to make bakeware. The safety of silicone bakeware has been the subject of various studies and are known to contain substances of toxicological concern that can migrate into food[2]personal care products, coatings and many other products. As a consequence of their wide use, VMS can be found in different environmental media, as well as in humans. We bought 14 new silicone baking moulds and 3 metallic moulds from the market and used them in different baking experiments. Four of the silicone baking moulds were produced in Germany, two in Italy, four in China, and for the other moulds were no information available. The metal forms were all produced in Germany. ⇨

VMS were measured in the indoor air throughout the baking process and at the edge and in the center of the finished cakes using a GC/MS system. Additionally, the particle number concentration (PNC, including octamethylcyclotetrasiloxane (D4), decamethylcyclo-pentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6), which are on the ECHA Candidate List of Substances of Very High Concern (SVHC) due to their properties as persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB). Moreover, D4 is classified as toxic to reproduction, category 2, according to CLP.

2) Food packaging and textiles

PFAS have been widely used in food contact materials, such as microwave popcorn bags, pizza boxes and fast-food containers, to prevent grease and oils from leaking. Since PFAS are also resistant to water, they have been applied to outdoor clothing and carpets. The solutions for food packaging and textiles overlap. Currently the alternatives include natural oils and waxes as well as the use of cellulose-based materials. Natural oils and waxes can also replace PFAS in dental care products such as dental floss.

3) Personal care products

PFAS have been used in some personal care products, such as dental floss[3], foundations, liquid lipsticks, and waterproof mascaras[4], to make them water-resistant. As with packaging and textiles, natural oils and waxes can also replace PFAS. Candelilla wax is an example of such a substitute that is used in personal care products.

4) Electronics

PFAS are also used to coat phone and tablet and monitor screens and even in circuit boards, cabling and wiring. to make them water-resistant. The extensive use of PFAS in electronics relates to their water repellency but also dielectric (insulation) properties, flame resistance and resistance to corrosion. Here fluorine-free replacement chemicals can be used such as polyurethan and silicone, however, they are seen as inferior solutions due their lower water repellency, thicker coat required and longer drying times[5].

5) Fire-fighting foams

Class B fires involve flammable liquids or flammable gases. Class B (and class A/B) fire-fighting foams include aqueous film-forming foam (AFFF). The use of certain PFAS in AFFF foams is widespread as these substances act as surfactants that help to spread the foam rapidly over the fire, thereby enhancing the fire-fighting effect. Alternatives that have been developed include fluorine-free silicone surfactant-based fire fighting foams.

II – DEGRADATION TECHNOLOGIES

As reviewed by Meegoda et al., some of the technologies that are available to break down PFAS are electrochemical oxidation, plasma, photocatalysis, sonolysis, supercritical water oxidation, and thermal degradation/incineration[6].

1) Electrochemical oxidation

This method uses an electrical current to oxidize and reduce organic pollutants. It can break down PFASs into harmless products with the creation of oxidants and the defluorination of intermediate products. Long-chain PFASs can be removed with high efficiency, but short-chain PFASs may be more difficult to degrade and could even increase in concentration due to precursor conversion.

2) Plasma

Plasma is an electrically charged gas produced by adding energy that ionizes gas molecules. In plasma-based water treatment, the electrical discharge between two electrodes in the vicinity of liquid water creates highly reactive oxidative and reductive species. PFAS molecules adhere to the water bubble interface, where the charged portion of the PFAS molecules interacts with the high energy ions in the plasma state.

3) Photocatalysis

Photocatalysis involves activating a substance with a photocatalyst, such as titanium dioxide, in the presence of photons (UV or visible light) to accelerate the destruction of organic contaminants in water and air.

4) Sonolysis

Ultrasound waves induce chemical reactions that degrade water contaminants such as PFAS. The process involves the generation and collapse of vapor bubbles, generating high temperatures that convert water vapor into reactive radicals (H- and OH-) which then degrade organic contaminants. ⇨

5) Supercritical water oxidation

Supercritical water solutions are kept at high temperatures and pressures above the critical point of water, where the fluid is neither liquid nor gas, but has properties of both. This state enhances organic solubility and oxidation, making it useful for breaking down compounds like PFASs that are difficult to oxidize under normal conditions.

6) Thermal degradation/incineration

Incineration is a method of treating materials by applying heat to decompose or destroy them. Hazardous waste incineration is typically carried out in an oxygen-rich atmosphere at 980 °C to 1200 °C, resulting in almost total destruction of organic compounds, including PFAS.

III – CONCLUSION

The pressure is mounting and PFAS appear to be well on their way towards substitution. Alternatives have already been developed but the biggest obstacle is likely the change of mindset and expectations. We have grown accustomed to the excellent water and oil repellency characteristics of PFAS but we will perhaps need to be content with the very good water and oil repellency of the alternatives. In some applications, substitution will be more challenging and essential uses will likely remain until feasible alternatives are found. Once PFAS are all but out of sight, the focus will need to shift towards their elimination form our environment.

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Health Effects of PFAS – Do We Even Know?

By *Marcha Verheijen*

PFAS (perfluoroalkyl and polyfluoroalkyl substances) consist of chains of carbon and fluorine atoms that create an incredibly strong chemical bond, rendering them resistant to breakdown. Although US companies no longer produce legacy PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), thousands of other PFAS are still being manufactured[1]. These substances persist in both the environment and the human body, with their serum half-life ranging from 44 days (PFPeS) to approximately three years (PFHxS & L-PFOS)[2].

The websites of the CDC/ATSDR (Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry)[3] and the EPA (U.S. Environmental Protection Agency)[4] report that exposure to high levels of certain PFAS may lead to:

1) Increased cholesterol levels, changes in liver enzymes and/or risk of obesity.
2) Interference with the body's natural hormones.
3) Reproductive effects such as decreased fertility or increased high blood pressure/pre-eclampsia in pregnant women.
4) Developmental effects or delays in children, including low birth weight, accelerated puberty, bone variations, or behavioral changes.
5) Increased risk of some cancers, including prostate, kidney, and testicular cancers.
6) Reduced ability of the body's immune system to fight infections, including reduced vaccine response.

The severity and impact of many of these health effects on society are straightforward. However, the effect of PFAS on the immune system may not seem so concerning at first glance. While the consequences of altered immune function may be subtle for an individual, such as catching an extra cold or taking slightly longer to recover from an infection, at a population level, there could be an economic impact in terms of increased doctor visits and people taking time off work for their own sickness or to care for sick children[1].

But a reduced response to vaccines could bring additional issues to our society. We all saw during the COVID pandemic what impact a virus can have on our society when we are not well protected. Imagine what other outbreaks could happen when the vaccines we are so custom to, do not work properly anymore. Take for example DTaP vaccination against Diphtheria, Pertussis, & Tetanus or MMR vaccination against Measles, Mumps, & Rubella. In 2012, Professor Grandjean[5] re-analyzed a collection of blood serum samples from fully vaccinated children, which was previously used to track PFAS levels, to investigate antibody levels after PFAS exposure. Shockingly, at age 5, more than one-quarter of the children were below the protective level for tetanus, and more than one-third were below the protective level for diphtheria. A more recent meta-analysis[6] reported a stronger association with PFOA, PFOS, and PFHxS with immunosuppression, as opposed to PFNA or PFDA and that the association was stronger for Diphtheria, Rubella, and Tetanus antibodies compared to other types of antibodies. ⇨

Looking at the health effects that have been reported so far for PFAS, we are aware that there might be a real health threat. It is not without reason that the legacy PFAS (PFOA and PFOS) were put on the list of persistent organic pollutants (POPs) by the Parties of the Stockholm Convention in 2009. As a result, manufacturers began to replace long-chain PFAS with short-chain PFAS. It is often assumed that short-chain PFAS can cause similar or lesser effects than PFOA/PFOS[7]. The key word here is assumed. This transition might actually turn out to be a regrettable substitution, because the biggest problem is that there is still so much uncertainty. That is also very obvious when reading the CDC/ATSDR and the EPA websites. They spend just as much text explaining PFAS health effects as they do on emphasizing the uncertainty that goes along with scientific research. They for instance include that the variety of reported health outcomes can be due to differences in participants, exposure type and investigated type of PFAS. But also, that research is still ongoing to understand the health effects of different dose levels, types PFAS, and chronic exposure to low levels of PFAS (especially in children).

The potential risks to public health posed by these “forever chemicals” are still being investigated by scientists. Currently, a majority of the toxicity data on PFAS are based on a few chemicals (including legacy PFAS), while toxicity data for hundreds of other PFAS currently in use are absent[8]. Similar to other chemicals, PFAS have the potential to cause a wide range of harmful health effects, depending on the circumstances of exposure (such as exposure magnitude, duration, and route) and individual factors (such as age, sex, ethnicity, health status, and genetic predisposition)[8]. Overall, many questions remain. Scientists don’t know the toxicity levels of many PFAS or how mixtures of PFAS may interact to affect human health[1].

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The State of Affairs of PFAS Regulation

Concerns about the potential health and environmental impacts of PFAS (per- and polyfluoroalkyl substances) have been growing in recent years. PFAS are persistent in the environment and can bioaccumulate in living organisms, including humans. Studies have linked PFAS exposure to a range of health effects. To address these concerns, regulatory bodies around the world have taken action to reduce or ban the use of certain PFAS chemicals. Below, some recent regulatory developments are covered, including some opportunities to comment on proposals.

By *Carolien Schophuizen*

The United States Environmental Protection Agency (EPA) has been actively studying PFAS and its potential impact on human health and the environment. The EPA has established health advisory levels for some PFAS in drinking water (70 ppb) and have been considering new regulations that would restrict the production and use of certain PFAS chemicals. On August 26, 2022, EPA issued a proposal to designate two of the most widely used PFAS (perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), including their salts and structural isomers) as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund. This rulemaking would increase transparency around releases of these harmful chemicals and help to hold polluters accountable for cleaning up the contamination that they cause. In practice, this requires entities to immediately report the release of PFOA and PFOS that meet or exceed the reportable quantity (For PFOA and PFOS, EPA has proposed an RQ of greater than or equal to one pound or 0.45359237 kg per 24 hours) to the National Response Center, state or Tribal emergency response commission, and the local or Tribal emergency planning committee (local emergency responders). A recent request for input and information was issued by EPA on April 13th. EPA is seeking input on whether to propose to designate additional PFAS, including HFPO-DA, known under the trade name GenX, and compounds that degrade in the environment by processes such as biodegradation, photolysis, and hydrolysis, to form certain PFAS. EPA is also seeking information on whether some PFAS compounds can or should be designated as a group or category. [3]

A Federal Register Notice has been published in the Federal Register at docket EPA-HQ-OLEM-2022-0922 and can be viewed on www.regulations.gov. The advanced notice of proposed rulemaking (ANPRM) will be open for a 60-day comment period through June 12, 2023.

The European Chemicals Agency (ECHA) has classified some PFAS chemicals as substances of very high concern (SVHCs) under the EU's REACH regulation, which triggers requirements for testing and risk management. The EU has also banned the use of certain PFAS chemicals in consumer products. On 13 January 2023, a PFAS restriction proposal was submitted to ECHA by authorities in Denmark, Germany, the Netherlands, Norway, and Sweden. It intends to reduce PFAS emissions into the environment and make products and processes safer for people. In the proposal a PFAS is defined as any substance that contains at least one fully fluorinated methyl (CF3-) or methylene (-CF2-) carbon atom (without any H/Cl/Br/I attached to it). Under this restriction, these PFAS should conform to the following conditions: They cannot be (1) manufactured, used, or placed on the market as substances on their own. In addition, (2) they cannot be part of another substance, as a constituent, a mixture, or an article in a concentration equal to or above:

i. 25 ppb for any PFAS as measured with targeted PFAS analysis (polymeric PFASs excluded from quantification)
ii. 250 ppb for the sum of PFASs measured as sum of targeted PFAS analysis, optionally with prior degradation of precursors (polymeric PFASs excluded from quantification)
iii. 50 ppm for PFASs (polymeric PFASs included). If total fluorine exceeds 50 mg F/kg the manufacturer, importer or downstream user shall upon request provide to the enforcement authorities a proof for the fluorine measured as content of either PFASs or non-PFASs.

(3) Paragraphs 1 and 2 shall apply 18 months from entry into force of the restriction. [2]

The six-month consultation on the restriction proposal, prepared by the Danish, German, Dutch, Norwegian and Swedish authorities, opens on 22 March 2023 and closes on ⇨

25 September 2023 (23:59 Helsinki time) and can be viewed on https://echa.europa.eu/nl/restrictions-under-consideration/-/substance-rev/72301/term.

Health Canada is considering activities that would address PFAS as a class. A notice of intent to address the broad class of PFAS was published in the *Canada Gazette*, Part I: Vol. 155 No. 17 – April 24, 2021.

Since 2021, Canada continued investing in research and monitoring on PFAS, to collect and examine information on PFAS to inform a class-based approach, and review policy developments in other jurisdictions. Furthermore, they intend to publish a state of PFAS report, which will summarize relevant information on the class of PFAS. [1]

Stakeholders and interested parties will have opportunities to provide input to help inform Government of Canada activities related to addressing PFAS as a class. As a first step, stakeholders are invited to provide initial feedback on the intent to address PFAS as a class, including challenges or opportunities they foresee, or indicate their interest in being engaged in future discussions by mailing substances@ec.gc.ca.

The **Australian government** has developed a national management plan for PFAS, which includes monitoring, research, and cleanup efforts. The PFAS National Environmental Management Plan (PFAS NEMP) provides nationally agreed guidance and standards on the investigation, assessment and management of PFAS wastes and contamination in the environment, including prevention of the spread of contamination. Developed by all State, Territory and the Australian Governments, as well as the New Zealand Government, through HEPA's National Chemicals Working Group, the plan recognizes the need for implementation of best practice regulation through individual jurisdictional mechanisms. It supports action on PFAS contamination around Australia and New Zealand. [4] The plan can be found here: https://haveyoursay.agriculture.gov.au/nemp-on-pfas

The **World Health Organization (WHO)** initiated the development of a background document for the Guidelines for drinking-water quality (GDWQ) on PFAS in drinking water with a focus on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). A draft background document was offered for public consultation from 29 September to 11 November 2022. The draft document was not received well, as it was thought not to be protective enough to human health. In a letter, (November 2022) 116 scientists stated, "We strongly recommend that this document be significantly revised and the numerous peer-reviewed scientific studies demonstrating strong links between PFOS and PFOA exposure and the many adverse health outcomes be carefully considered. Otherwise, the proposed guidance should be withdrawn." [5] In total, WHO received 25 sets of comments from different stakeholders and is currently consolidating and reviewing their feedback, with the view to developing guideline values for PFOS and PFOA.

The Stockholm Convention on Persistent Organic Pollutants (POPs) is a global treaty aimed at protecting human health and the environment from chemicals that are persistent, bioaccumulative, and toxic. The convention was adopted in 2001 and entered into force in 2004. One of the chemicals covered by the Stockholm Convention is perfluorooctane sulfonic acid (PFOS), a type of PFAS chemical. In 2009, the convention added PFOS and its salts, as well as perfluorooctanoic acid (PFOA) and its salts, to its list of restricted chemicals. Under the Stockholm Convention, countries are required to take measures to eliminate or reduce the production, use, and release of POPs, including PFOS and PFOA. This includes phasing out the production and use of these chemicals, as well as ensuring the environmentally sound management of stockpiles and wastes containing these chemicals. The Stockholm Convention has played an important role in addressing the global issue of PFAS contamination and in raising awareness of the potential health and environmental risks associated with these chemicals. While the convention specifically focuses on PFOS and PFOA, it has helped to spur action on other PFAS chemicals as well [7]. The Stockholm Convention on persistent organic pollutants are implemented in the EU by means of Regulation (EC) No 850/2004 (the 'POP Regulation'). A summary is provided below [6]:

- PFOS: since 2009, perfluorooctane sulfonic acid and its derivatives (PFOS) have been included in the Stockholm Convention to restrict their use. PFOS has then been restricted under Annex I of the EU's Persistent Organic Pollutants (POPs) Regulation. Annex I entry for PFOS was amended in 2020 to remove exemptions no longer needed in the EU.
- PFOA: since 2019, perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds have been included in the Stockholm Convention to eliminate their use. PFOA has been banned under the POPs Regulation since 4 July 2020.
- LC-PFCAs: in 2021 Canada proposed to consider long-chain perfluorocarboxylic acids (C9-C21 PFCAs) for inclusion in the Stockholm Convention
- PFHxS: Since 2022, perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds have been included in the Stockholm Convention to eliminate their use. Consequently, PFHxS will be restricted in the EU by the POPs regulation. Formal inclusion is planned in the first half of 2023. ⇨

California's PFAS regulation: In California, a ban on firefighting foam containing PFAS is one of the first examples of action taken to address PFAS contamination. California has also taken broader action to address PFAS contamination. For example, in 2020, the California State Water Resources Control Board adopted an order requiring certain industries to test for PFAS chemicals in their wastewater discharges, and PFAS levels are monitored in public water systems. In 2023, the State of California prohibited the distribution and sale of food packaging containing regulated PFAS chemicals. Manufacturers are required to include a list of intentionally added PFAS chemicals on their website from the beginning of this year, and will need to add it on product labels by 2024. On July 1, 2023, the California state will prohibit the distribution and sale of products containing PFAS, designed for use by infants and children under 12 years of age. Manufacturers should apply the least toxic replacement chemical as an alternative to PFAS substances. In 2025, two more pieces of regulation will come into force. The first prohibits the use of PFAS substances in textile articles. The second prohibits the presence of PFAS in cosmetics. [8]

If certain PFAS chemicals were to be banned or restricted, it could potentially affect industries, as companies may need to reformulate products or find alternative materials. Some companies are already investing in research and development to develop safer alternatives to PFAS, and many stakeholders are calling for more action to address the issue of PFAS contamination. The outlook for industry will depend on the specifics of any regulations that are put in place.

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Proposal for an EU/EEA-wide Restriction on PFAS

By *Bureau REACH, RIVM*

On 7 February 2023, a proposal for an EU/EEA-wide restriction on per- and polyfluoroalkyl substances (PFASs) was published by ECHA, the European Chemical Agency in Helsinki. The aim of the restriction proposal is a future ban on the manufacture, use and placing on the market (which includes import) of PFASs as substances on their own, as a constituent in other substances, in mixtures and in articles. This ban is meant to minimize emissions, thereby limiting exposure of humans and the environment and thus limiting the risks of exposure to this group of substances with a very high persistence. The Netherlands (Bureau REACH of the National Institute for Human Health and the Environment) collaborated for this proposal with Denmark, Germany, Norway and Sweden to draft and submit the proposal under the EU chemicals regulation REACH. The proposal does not yet constitute an official piece of legislation as it is still subject to a scientific review process by ECHA's scientific committees and finally a decision-making process involving the European Commission, Member States and the European Parliament. Details of the proposed restriction are published on the website of ECHA[1].

Preparation of the Restriction Dossier

The OECD PFAS-definition is based on chemical structure. Hazardous properties or risk considerations are not part of it. The substance scope of the proposed restriction is additionally a concern-based one as it only intends to cover PFASs that are persistent. All PFASs and/or their degradation products that are in the scope of this restriction proposal share the same concern: the very high persistence, exceeding the persistence observed for any other chemical class, i.e., the focus of the restriction proposal lies on the 'forever chemicals'. The absolute majority of substances covered by the OECD PFAS-definition share this concern and are thus included in the scope of this restriction proposal. A broad scope is used in order to avoid one PFAS being replaced by another that is associated with a comparable hazard – so called "regrettable substitution". Grouping of substances to speed up regulation is in line with the strategies of ECHA and the European Commission, as a goal of the Chemicals Strategy for Sustainability (CSS), which is part of the EU Green Deal. The proposal might have a significant impact on a high number of industry sectors and companies. Given the trade links with other parts of the world and the relevance of the proposal for imports, the proposal might have consequences beyond the borders of the EU Member States.

Restrictions under REACH can be initiated by authorities, and they have to provide evidence for a unmanageable risk of a (group of) substance(s) in a so-called Annex XV restriction dossier. Preparing a restriction proposal is a time-consuming and intensive process, requiring the involvement of experts from a wide variety of disciplines such as chemistry, (eco)toxicology, and economics. Given the scope of the restriction proposal, which ECHA describes as one of the broadest in the EU's history, and the high number of uses that had to be mapped, collaboration with colleagues from five EEA countries was of paramount importance. The work was split up into multiple work packages and involved a Risk Management Option Analysis (RMOA), as well as separate work packages responsible for developing the appropriate chemical scope, assessing the hazards associated with PFASs for humans and the environment, mapping and describing the uses of PFASs, generating an overview of analytical methods available per use, describing the current state of presence in humans and the environment, e.g. based on monitoring data, and assessing the positive and negative impacts associated with a restriction on the use of PFASs. In the RMOA, potential regulatory management options to control the risks associated with exposure to PFASs are de-

scribed and discussed. A restriction was ultimately ⇨ considered to be the most effective measure. The concern/hazard has to be described in detail as this is the foundation of the restriction and essential for justifying why regulatory action of this group of substances is needed at an EU-wide level. Collecting data on uses is important as it provides crucial insights into the volumes used as well as associated emissions, and whether alternatives are already on the market or under development. As such, it presents the basis for ultimately assessing the costs and benefits associated with a restriction. Fourteen different use sectors were investigated in detail. Examples are food contact materials, cosmetics, electronics, and textiles. The assessment of analytical methods is important for ensuring that the proposed restriction is enforceable. Assessing the costs and benefits associated with a restriction in the socio-economic analysis is crucial for developing a well-balanced and proportionate proposal that avoids unintended and unwanted impacts, by determining applications for which derogations are needed to, for example, avoid the unavailability of certain products in the EU due to a lack of alternatives that affected companies can substitute to or other challenges, such as high investment costs or supply shortages for raw materials, associated with substitution.

To help with gathering the required information, two consultations were held, with the first one – the so-called "Call for Evidence" launched in the summer of 2020. A second consultation started in summer 2021. Use-specific reports summarizing the existing knowledge base of the Dossier Submitters were made available and stakeholders were, for example, asked to comment on tonnages, emissions, (applicability of) alternatives and costs. Nevertheless, important knowledge gaps remain. For several applications, the substitution potential is, for example, still unclear. Consultation with stakeholders therefore remains an important part of the regulatory process and for finalizing the restriction.
To allow for a thorough analysis of all information that came in through the consultations, the submission of the dossier was postponed by six months, resulting in the submission of a dossier exceeding 1800 pages on 13 January 2023, with a pre-publication by ECHA on 7^{t} of February and the official publication on 22 March. The gathering of information and drafting of the dossier took about 2.5 years and numerous people from the five countries were involved.
With the publication, stakeholders have the chance to read the proposal and comment on it during a six-month consultation closing on 25 September 2023. The Dossier Submitters and ECHA have drafted specific information requests to help stakeholders with identifying the type of information that is still needed. Comments can range from commenting on omissions, e.g. highlighting uses of PFASs that were not known to the Dossier Submitter and bringing forward specific data on tonnages and emissions for specific uses to additional information on the availability and feasibility of alternatives.

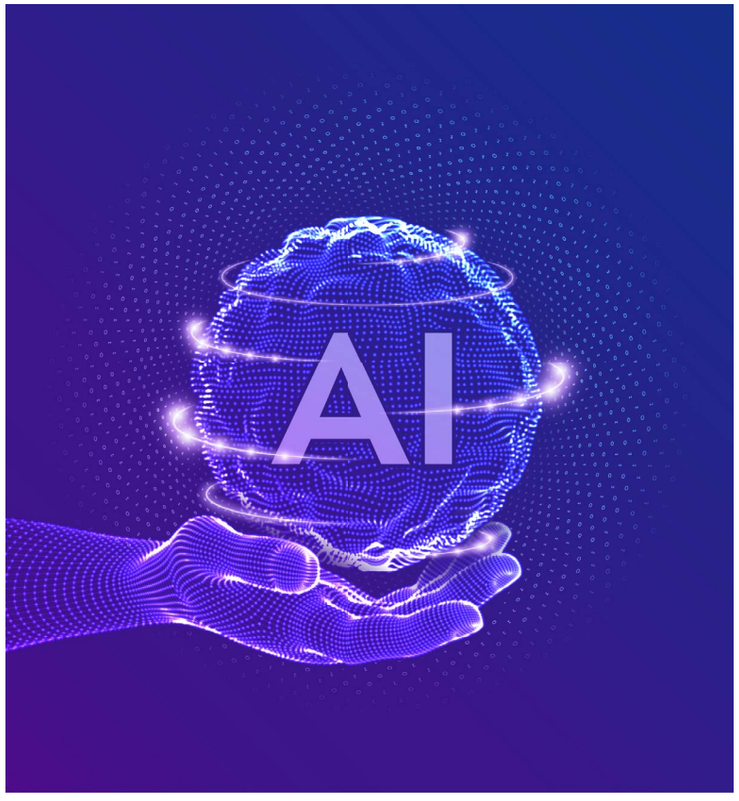
The proposal is now under scrutiny by the two scientific committees of ECHA, consisting of independent experts: the committee for risk assessment (RAC) and the committee for socio-economic analysis (SEAC). The information that is submitted by stakeholders in the coming months will be processed by ECHA's scientific committees and the Dossier Submitters. The opinion of the scientific committees may differ from the original proposal, e.g. suggest changes to derogations. Based on the opinions from RAC and SEAC, the European Commission will draft the legislative text that is scrutinized by the Member States before adoption.

[1] https://echa.europa.eu/nl/-/echa-publishes-pfas-restriction-proposal

ToxAIcology - How Modeling and AI Could Be Used for Risk Assessment

By *Thomas Hartung*

In 2022, the International Conference of Toxicology in Maastricht, EuroTox and SoT had their traditional debate, this time on “Is there a role for artificial intelligence and machine learning in risk decisions?”. I had the privilege to represent EuroTox while my dear colleague Craig Rowland spoke for SoT, with reversed pro/con roles compared to SoT in San Diego earlier that year. Today, and probably already last year, the question should have been “Is there a role for risk decisions without AI?”. All of science embraces AI; how could toxicology stay out? In 2021, already 2.5% of all scientific publications include AI. Stanford’s Artificial Intelligence Index Report estimated the total number of AI publications at half a million. The respective AI industry is growing with 38% CAGR (Compound Annual Growth Rate).



The gain in AI capability is most impressive: For illustration, on 14 of March this year, GPT-4 was released: GPT-4 performed at the 90th percentile on a simulated bar exam (for lawyers), the 93rd percentile on an SAT (the US standardized test done at the end of high school) reading exam, and the 89th percentile on the SAT Math exam. Whatever you think of US high school education or lawyers, that is quite impressive (GPT-3.5 released one year earlier was around the 10th percentile for these). Do you really think that doing risk assessments is more demanding?

AI, aka machine learning, has become one of the most disruptive technologies due to the synergy of growth in computational power, growth in available “big” data, and optimization of machine learning. To put some numbers behind this: Our computers have doubled in computational power every two years (at half the price), known as Moore’s law – Gordon Moore, the cofounder of Intel, who passed away in March, stated this in 1965 and the prediction held essentially until today. The data in the world are estimated to increase currently about 60% per year or, expressed another way, 85% of all data in the world were produced in the last four years. Last, since deep learning achieved in 2012 human-level performance on certain pattern-recognition tasks, AI has doubled in capacity every 3 months; this is quite impressively shown by GPT-4 trained on about one million more parameters than GPT-3 in 2020. Together, the increase in AI power is more than one billion-fold over the last 50 years.

This affects all areas of modern life, including toxicology. Information retrieval and data extraction are obvious examples. We are not far from computers being able to read scientific papers with the quality of a student, but not one per week but millions at a time and without forgetting anything. Challenges are at this moment mainly tables and figures, but that is a matter of time only. Digital pathology with image analysis and sharing impacts on toxicology. Big data in toxicology originate from increasingly curated legacy studies, the scientific literature becoming open ⇨

and machine-readable, the grey literature of the internet (e.g., more than 900,000 safety data sheets), sensor technologies, high-throughput testing (such as ToxCast and Tox-21), and ~omics technologies. We have come far from counting dead animals…

The big challenge for modern toxicology is this multitude of information sources. AI uniquely lends itself to evidence integration. In 2018, we showed that an automated read-across (RASAR, i.e., read-across-based structure activity relationship), achieved 87% balanced accuracy for nine OECD guideline tests with 190,000 chemicals with results in a five-fold cross-validation. In the same study, six of the OECD tests showed on average only 81% reproducibility (Luechtefeld et al., 2018). While humans can only handle a limited number of parameters, AI thrives on Big Data – noteworthy, Big Data is defined by the 3V of volume, variety (of data types) and velocity (of data becoming available). The output of AI-based data analysis is probabilities, which opens opportunities for AI-enabled probabilistic risk assessment. The EU ONTOX project (Vinken et al, 2021) explores many of the opportunities from Natural Language Processing for data extraction to predictive toxicology based on probabilistic risk assessment. In this context, we just created a database of 200 million triplets of a chemical / property / result, a treasure trove for computational toxicology.

At the same time, we should not see AI as doing just what a human can do on a larger scale. In 2022, DeepMind's chess program AlphaZero showed that without training on any human game of the past, it matched the world champion Magnus Carlsen's ELO ranking of 2882 points after about four hours and reached unbeatable 3581 ELO points after eight hours. Most interestingly, the software is playing chess differently with unseen strategies, to an extent that human players have started studying its way of playing to improve theirs. We should not only expect AI to do our job but also to find new ways in doing things.

Altogether, AI represents challenges and opportunities for toxicology. For regulatory purposes, the black-box character of many methods is difficult to accept but explainable AI (AIx) is rapidly developing; at this moment, AIx predictions are less accurate, but this might change. A big problem are biases in datasets, but if we can find the bias, the machine can as well… Certainly, humans must be in the loop, but AI offers condensed information on a silver platter. User interfaces become easier and easier. Still, we must be worried about the AI literacy of students and practitioners on both sides of the regulatory divide. It should not be possible these days to complete a degree in toxicology without some training and familiarity with AI. Let's start to develop the curricula and embrace ToxAIcology – it is coming, whether we want it or not.

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Ohio Train Derailment Raises Toxicity Concerns

EPA and Justice Department File Complaint Against the Railroad Company Over Environmental Damage

By *Barae Jomaa*

On the 3rd of February, 2023, a Norfolk Southern Railway Company train carrying hazardous materials derailed in East Palestine, Ohio, causing a fire and damage to the environment. The derailment also led to the contamination of air, land, and water in the area, which led to the evacuation of residents living near the site. Following the incident, the US Environmental Protection Agency (EPA) and the Department of Justice filed a complaint against Norfolk Southern Railway Company over the environmental damage caused by the derailment[1].

The spill, and associated risk to human health and the environment, has been widely covered by global media organizations[2–4].

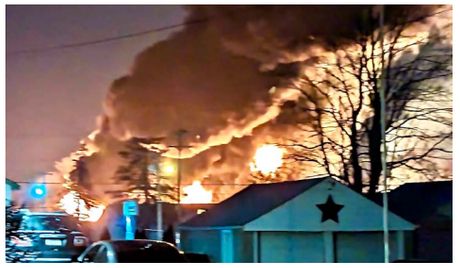
The complaint filed by the US EPA and the Justice Department alleges that the railway company violated the Clean Air Act, the Clean Water Act, and the Resource Conservation and Recovery Act. The complaint also contends that Norfolk Southern failed to take adequate measures to prevent the derailment and failed to respond adequately to the incident, resulting in additional environmental damage.

According to the US EPA, the hazardous materials carried by the train included vinyl chloride, ethylene glycol monobutyl ether, ethylhexyl acrylate, butyl acrylate, isobutylene, and benzene residue. The derailment caused a pile of burning rail cars, and as the fire burned, the temperature in one of the rail cars containing vinyl chloride began to rise. In effort to avert an explosion, Norfolk Southern burned five rail cars that contained vinyl chloride. Burning vinyl chloride is known to release carbon monoxide and carbon dioxide as well as the corrosive substances hydrogen chloride and phosgene.

The US EPA and other US federal agencies immediately responded to the situation, providing on-the-ground assistance to first responders and conducting extensive testing in and around East Palestine. As of March 29, 2023, 9.2 million gallons of liquid wastewater had been shipped off-site, and around 12,932 tons of contaminated soil and solids had been shipped off-site[1].

Following the incident, the US EPA issued a Unilateral Administrative Order to Norfolk Southern Railway Company, requiring the company to take actions to address the environmental damage that was caused by this derailment. The US EPA has been overseeing Norfolk Southern's work since the issuance of the order.

Disastrous derailment: Drone footage shows the freight train derailment in East Palestine, Ohio, U.S., February 6, 2023 in this screengrab obtained from a handout video released by the NTSB. Image courtesy of National Transportation Safety Board.



Toxic plume: Smoke from the accident, on February 3. Image courtesy of thunderlips36.

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AIO toxafette - Damian Roelofsen

In the toxafette, PhD-students working in the toxicology field get the chance to share their experiences in performing research. Every issue a new candidate answers a series of questions, and then passes the baton to a fellow PhD-student. This time Damian Roelofsen, from Radboud University in Nijmegen tells us about his project.

Can you introduce yourself?
My name is Damian Roelofsen, I was born and raised in Arnhem, quite close to Nijmegen. At the Radboud University in Nijmegen I did both my bachelor and Master biomedical Sciences. In September last year, I started working at the department of Pharmacy, division of Pharmacology and Toxicology. I currently work as a junior researcher, but I will continue as a PhD-candidate after my year as junior researcher is finished.

How would you explain the subject of your research to a layperson?
We are looking at how much of a specific compound goes through the placenta. In other words, how much from a compound present in the blood of the mother will end up in the blood of the foetus. This concept can be studied in different ways. One way is by using whole placentas. We obtain them from women who give birth at the Radboud university medical centre (Radboudumc). Another way to study placental transfer is by using a cell line representing a specific type of cells present within the placenta. Currently, I am working on both these techniques in order to compare them and use them in combination with so-called physiologically based pharmacokinetic modelling. This is a computer model describes multiple processes that a compound undergoes within the human body. These models can be used to estimate exposure in "healthy" humans, but also in pregnant women.

How is your research related to the field of toxicology, and why did you choose this subject?
By assessing maternal and especially foetal exposure, we can combine this with data on concentrations at which adverse effects are observed, and in this way derive safety margins. With this approach, we try to develop a workflow by combining multiple *ex vivo, in vitro,* and *in silico* methods to estimate risks without the use of animals. I chose this subject because I did my first master internship at the department of Pharmacology and Toxicology at the Radboudumc and I became really fascinated by the combination of experimental lab work and computational modelling to assess exposure for mother and especially for the foetus.

What was your motivation for starting a PhD program?
As I followed my bachelor and master, I slowly found out how much I liked to perform research in the lab. I specifically chose some lab-oriented courses already during my bachelor, which I really liked. Also, during my internships in academia, I could confirm for myself that after my study I wanted to go into research. When a vacancy opened up from the department where I did my master internship, I immediately applied and got the job. After that, it was great news that I could stay to do a full PhD program.

How do you see the future of your research topic (follow-up research / social impact)? What do you hope for?
I think the combination of *in vitro* and *ex vivo* techniques with *in silico* modelling will become increasingly important for risk assessment and will also continue to find its way into clinical practice, for example in dosing advice for pregnant women. I believe this approach, in the end, may replace part of the research on animals. I hope this approach can help to protect the unborn child from risks now and in the future. Since it is difficult to study this population of pregnant women and ethically almost impossible to gather any information on the foetus in an invasive way, this approach can become a good alternative to protect the foetus as best possible against adverse effects from compounds to which pregnant women are exposed either voluntary (drugs) or involuntary (chemicals).

What goals do you have regarding your career after finalization of your PhD? Would this be inside or outside academia, and why? Would you consider going abroad?
Since I started quite recently, I have not given it much thought yet. I am not sure whether I would like to stay in academia or not. I think the main thing is that I would like to continue in the field of toxicology, whether that is in academia or for example in industry would be less of an issue for me. Going abroad is something I would consider, but it is not a must.

Does the project meet your expectations, why or why not?
I think the project definitely meets my expectations. I was hoping to spend a lot of time in the lab performing experiments and combine this data with computational modelling. This way I felt like I could gain experience in both *in vitro* and *in silico* research. I feel that this is exactly what I am doing now which gives me a lot of positive energy, although sometimes also the experimental work can be challenging (which brings me to the next question). ⇨

What is the biggest challenge for you in doing PhD research?
As stated, experimental work can give quite some challenges. Sometimes you think you have everything figured out, but then new difficulties arise. I find it sometimes difficult to cope with them in a good way as sometimes the doubt kicks into play. Then I start questioning everything (including myself) which is not the best way to cope with these problems. I think my PhD-project will challenge me in my personal development to cope differently with such things, which I think is also very valuable. I think it is good to not only perceive PhD-research as a professional development, but also as an opportunity to develop on a more personal level.

Would you consider qualifying yourself as European Registered Toxicologist (ERT), why or why not?
Yes! I would definitely like to qualify myself as ERT. Since I would like to continue in this field, I think it is a valuable professional development to pursue as it may be helpful for my next career step(s) after obtaining my PhD. During my masters here in Nijmegen I also followed a lot of toxicology courses and therefore I think it is a very logical step to take.

How do you expect society will benefit from your PhD-research?
I think that, ultimately, pregnant women and their unborn child will be better protected due to the research that I am currently conducting. I believe that continuing and extending this type of research is, and will be, beneficial for pregnant women. This research can prevent harm as small safety margins may lead to increased caution for certain compounds during pregnancy. Especially when you keep in mind that for many compounds there is almost no information regarding pregnancy.

Please answer the question from the last toxafette PhD-candidate: What is something you would have liked to know about doing a PhD-project, back when you were still a student?
I would have liked to know is that it is doable to conduct a PhD-project. Back then, I always thought that a PhD-project would be extremely difficult and stressful. And, although it sometimes is quite stressful and sometimes you are quite busy, it is not that I need to work all the time. So maybe it would have been great to know this beforehand, since for quite a time this image of a PhD-project scared me off a bit from pursuing it.

Could you propose a question to the next PhD-candidate?
My question would be: do you feel that you are conducting PhD research that will be an advantage to your career after you are finished with your PhD?

REGISTRATIE CIE

Voorletters	Achternaam	Opleider	Datum inschrijving
T.J.	Meuwissen	Prof.dr.ir. I.M.C.M. Rietjens	28-03-2023

What If All Our Products Containing PFAS Would Disappear Tomorrow?

It is interesting to think of a world where PFAS are completely restricted without any exception and all products containing PFAS would disappear tomorrow. Stopping the production completely would first induce a reduction of greenhouse gasses. Obviously, it would also stop the accumulation of PFAS in human bodies and the ecosystem. But what about our daily life? What would it change?

By *Héloïse Proquin and Marcha Verheijen*

As said before (in the article "PFAS alternatives and degradation technologies"), many daily products have been produced with some PFAS in them. The list of products containing PFAS is very long and goes from non-stick cookware to fire retardants, including stain and water repellents, some furniture, waterproof clothes, pizza boxes and take-out containers, food packaging, carpets and textiles, rubbers and plastics, electronics and some cosmetics such as dental floss. Another important category where PFAS can be found is in pharmaceutical products.

A full ban of PFAS and the disappearance of the products would mean that we would not have many of our daily products as many are present in plastics and rubber. Imagine entering your house when you suddenly find all products containing PFAS removed.

First, most of the furniture containing plastics would be gone. Lights would be gone too, so we need candles to have some light but don't play with matches, everything can easily burst into flames because nothing has fire retardant properties anymore. No carpet or PVC floor, you can feel the nice cold touch of bare concrete. No more electronics in the house anymore, so no more watching Netflix or TikTok. Time to talk to each other? If you have nothing to say, you can start cooking but all your non-stick cookware would be gone. You don't have much food in the house, everything is spoiled by the loss of fridge and food packaging. If you prefer to leave the house on a rainy day to pick up a pizza, you will get soaking wet because you don't have a waterproof raincoat or an umbrella anymore. And you would have to carry the pizza in your hand because the box is gone. So, after burning your hand from holding the hot pizza, it would soon turn soggy in the rain.

With no PFAS around anymore, we might see some crazy fashion trends emerge as people try to make waterproof clothing out of anything they can find. Picture a world where people wear suits covered in bees wax or only leather or even hats made of aluminum foil! Then we are directly protected from aliens trying to read our minds!

With no PFAS around, the amount of waste we produce is drastically reduced but we would still have to dispose of what is left. We would need cotton bags, which would leak on your non waterproof clothes making you all dirty. So, after taking out the trash, you would have to take a shower, oh wait… where is the shower? If you are lucky, you would still have a bathtub left, but can you fill it with water because some of the pipes were made of plastic. Even if you manage to obtain water, you have no more cosmetic products unless you previously stole soap in a hotel.

In this new world, you better not get sick and need medicine because some medicines contain a PFAS group in the molecule. Being rushed to the emergency room is no option anymore because there are no cars, paramedics would need to come by bike or horse and carriage. The problem of traffic jams is completely resolved.

All in all, you are probably sitting on the bare floor in the dark by candlelight. Your house is pretty empty with not much to do and all you can do is talk to the ones living with you and maybe play an old-fashioned board game.

I hope these silly scenarios brought a smile to your face! Remember, even serious topics can be approached with humor and lightheartedness.

NVT Travel Grant Report

Society of Toxicology's 62nd Annual Meeting in Nashville, Tennessee, the United States March 19–23

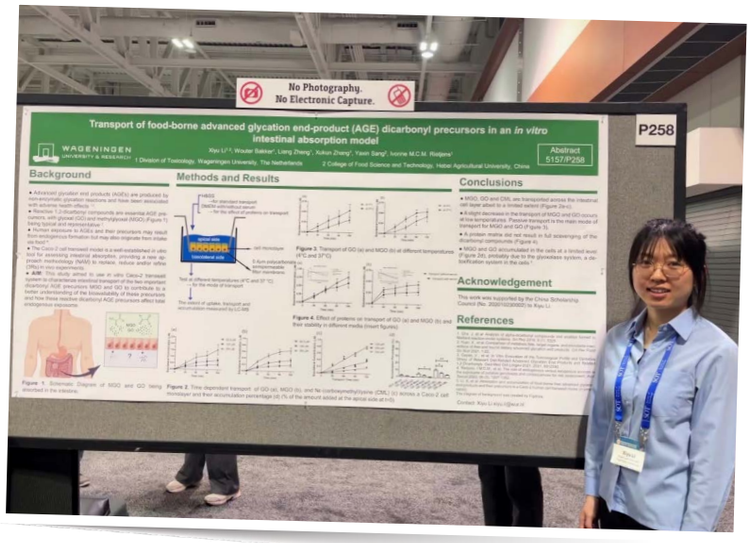
TRAVELER:
Xiyu Li,
Wageningen
University & Research

1. Describe what your presentation was about.

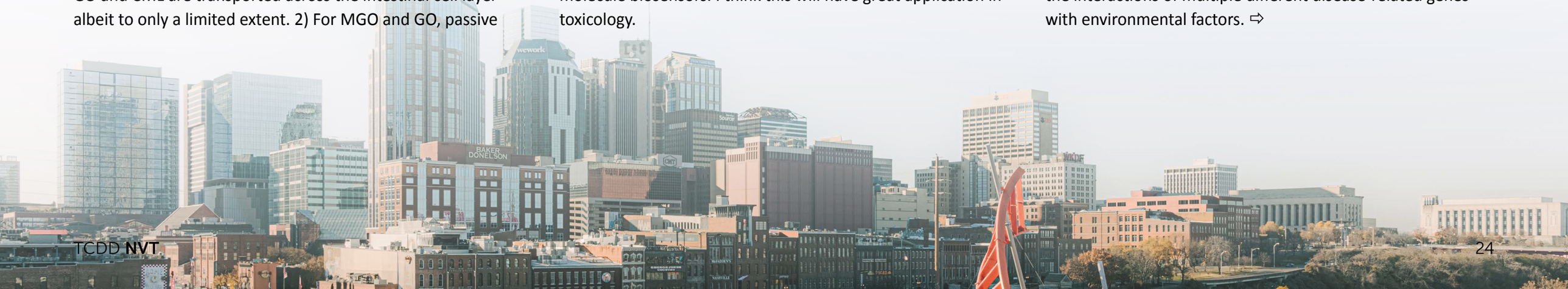
Advanced glycation end products (AGEs) are formed in the human body and in processed food through non-enzymatic glycation reactions. Reactive 1,2-dicarbonyl compounds, such as glyoxal (GO) and methylglyoxal (MGO), are important precursors of AGEs. The extent to which food-derived dicarbonyl AGE precursors contribute to endogenous exposure is currently unknown. Thus, this study aims to investigate how 1,2-dicarbonyl compounds like MGO, GO, and CML cross the intestinal barrier, using the Caco-2 transwell system in vitro. The study includes transport in protein-containing and protein-free media, at different temperatures, and non-cytotoxic concentrations. The results in this study showed that 1) MGO, GO and CML are transported across the intestinal cell layer albeit to only a limited extent. 2) For MGO and GO, passive transport is the main mode of transport. 3) The presence of a protein matrix did not result in full scavenging of the dicarbonyl compounds. 4) MGO and GO accumulated in the cells at only a limited level, probably due to the glyoxalase system, a detoxification system in the cells.

2. Describe the three most interesting learning/insights you took from the conference.

The presentation I thought was most interesting came from E. Lippmann of Vanderbilt University, who gave a talk on Engineered human Blood-Brain Barrier Models to understand molecular transport. Currently, in vivo BBB permeability studies are laborious and low throughput, and the accepted model of BBB permeability is the mouse, but it can not necessarily represent the BBB in humans (because of differences in expression and affinity of transport proteins). His presentation showed a solution developed by their team to differentiate induced pluripotent stem cells (iPSC) into BMEC-like cells and assemble them with other neurovascular cells into a microphysiological BBB model and some applications to small molecule biosensors. I think this will have great application in toxicology.



Another study that I found interesting originated from S Chen from FDI. They established a platform based on human pluripotent stem cells to study the effect of environmental toxins on the survival of human beta cells and dopamine neurons. Currently, determining the mechanism of gene-environment interactions has been a great challenge due to the lack of a suitable experimental platform. By using pancreatic ß-like cells derived from human pluripotent stem cells (hPSCs), they identified a common pesticide that induced pancreatic ß-cell death, an important pathological feature of diabetes. By screening different hPSC-derived cell types, they extended this observation to a similar sensitivity of midbrain dopamine neurons, a cell type that is affected in Parkinson's disease. Thus, hPSCs provide an extremely promising platform to study the interactions of multiple different disease-related genes with environmental factors. ⇨



With regard to the novel technologies, I would like to introduce the advanced and evolving single-cell technology. The field of single-cell technology is rapidly advancing and increasingly being applied in toxicology. By enabling the analysis of individual cells, this technology provides insights into how toxic substances interact with specific cell types, and improves our understanding of toxicity mechanisms and potential health effects. Moreover, single-cell analysis can reveal underlying variability in cellular responses to xenobiotics, offering a unique examination of efficacy, toxicity, and metabolism. Proteomics performed at the level of individual cells can comprehensively characterize heterogeneity in cell-to-cell xenobiotic outcomes, including drug-induced toxicity, which enhances progress toward personalized and precision medicine. Understanding toxicity at the single-cell level can also facilitate more informed translation of preclinical data.

3. Give your scientific "take home message" from this conference.
In vitro testing and predictive toxicology are promising emerging technologies that can enhance the field of toxicology, leading to improved risk assessment and management strategies.

4. Finally, describe in what ways the conference organization and yourself strived to make the conference a climate neutral event.

- Poster on recyclable paper, badges on paper to reduce plastic.
- Conference held in center committed to sustainability with green roof, water management, and other initiatives.

TRAVELER:
Jiaqi Chen,
Wageningen University & Research

1. Describe what your presentation was about.
To derive a point of departure (POD) for human health risk assessment upon acute neurotoxicity induced by saxitoxin (STX) exposure, a quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) approach facilitated with physiologically based kinetic (PBK) modelling was employed. Kinetic parameters describing oral absorption and hepatic biotransformation for model establishment were obtained using quantitative structure-activity relationship (QSAR) derivation and *in vitro* rat hepatocyte incubations. The results from the latter proved the limited metabolism of STX in liver, indicating that the clearance of STX depended mainly on renal excretion. PBK models were built for rats, mice and humans, with only glomerular filtration as a clearance process of STX, which could adequately predict the time profiles of blood and urine STX concentration as observed *in vivo*. Next, available concentration-based toxicity responses derived from *in vitro* rat, mouse and human cell models were collected, and were converted into *in vivo* dose-dependent datasets. Benchmark dose (BMD) analysis was then performed on these predicted *in vivo* curves to derive tentative PODs. Results show conservative POD predictions for rats and mice, while for humans the average prediction is comparable to the reported human no-observed-adverse-effect levels (NOAELs) derived by EFSA and the joint FAO/IOC/WHO ad hoc Expert Consultation. Moreover, interspecies ⇨

differences mainly due to toxicokinetics are found between humans and rodents (rats and mice), with humans are predicted to be more susceptible to acute neurotoxicity following STX exposure, which is in line with the toxicity data observed *in vivo*.

2. Describe the three most interesting learning/insights you took from the conference.

(1) Generalized concentration addition (GCA) model in mixtures analysis: GCA is a model for predicting joint effects of co-exposures to receptor ligands that vary in efficacy. It is firstly developed by Howard and Webster for calculating mixture effects of aryl hydrocarbon receptor agonists as an extension to the concentration addition (CA) model, considering that GCA is not restricted by the presence of chemicals with limited efficacy. GCA has been applied for estimating PPARa activation by PFAS mixtures, which is thought as an important molecular initiation event. Results show that GCA provides the best fit of data as compared to the other two approaches, relative potency factor (RPF) and effect summation, providing potential insights into using GCA for evaluating toxicity induced by PFAS mixtures.

(2) Role of population variabilities in human health risk assessment: A default value of 10 as an uncertainty factor is usually applied for taking population variability into account. However, this roughly estimated value might not be protective enough to all chemicals in terms of toxicokinetics and toxicodynamics. The bayesian meta-analysis of activities, tissue distribution and major polymorphic variants of phase I and phase II enzymes (i.e. CYP2D6, PON1, GST) has been conducting to further improve understanding in population-based toxicokinetic studies.

(3) Use of transcriptome-based POD (tPOD) in estimating a traditional (apical) POD: The promising use of tPOD for risk assessment is based on a hypothesis that a POD based upon comprehensive molecular data will be protective of any downstream apical effect POD, since all apical effects result from a prior change at the molecular level. A tPOD could be derived from whole-transcriptome data, and it is promising in replacing long-term exposure *in vivo* studies and in reducing animal use.

3. Give your scientific "take home message" from this conference.

Induced human pluripotent stem cells are promising in developmental (neuro)toxicity studies.

4. Describe in what ways the conference organization and yourself strived to make the conference a climate neutral event.

(1) Admission tickets for all conference activities are embedded in the code appearing on the back of a badge for every participant, to ensure that no paper ticket is needed.

(2) During the conference, I used a recyclable water bottle every day instead of the disposable plastic cups provided by the conference organizer.

I was happy to attend the SOT meeting held from March 19th-23rd 2023 in Nashville, truly the city of music, and I would like to thank the NVT for contributing to my travel.

During the meeting, I presented our data on the neurotoxic hazard characterization of fumes deriving from engine oils and hydraulic fluids used in airplane engines. Both engine oils and hydraulic fluids can leak into the ventilation system of airplanes using a bleed air system to supply the cabin with fresh air. Severe contamination, known as fume events, are rare, however, low-level contamination may occur more frequently, and several crew members reported neurological health complaints following experiencing a fume event. Nevertheless, there is limited data on the neurotoxic hazard of ⇨

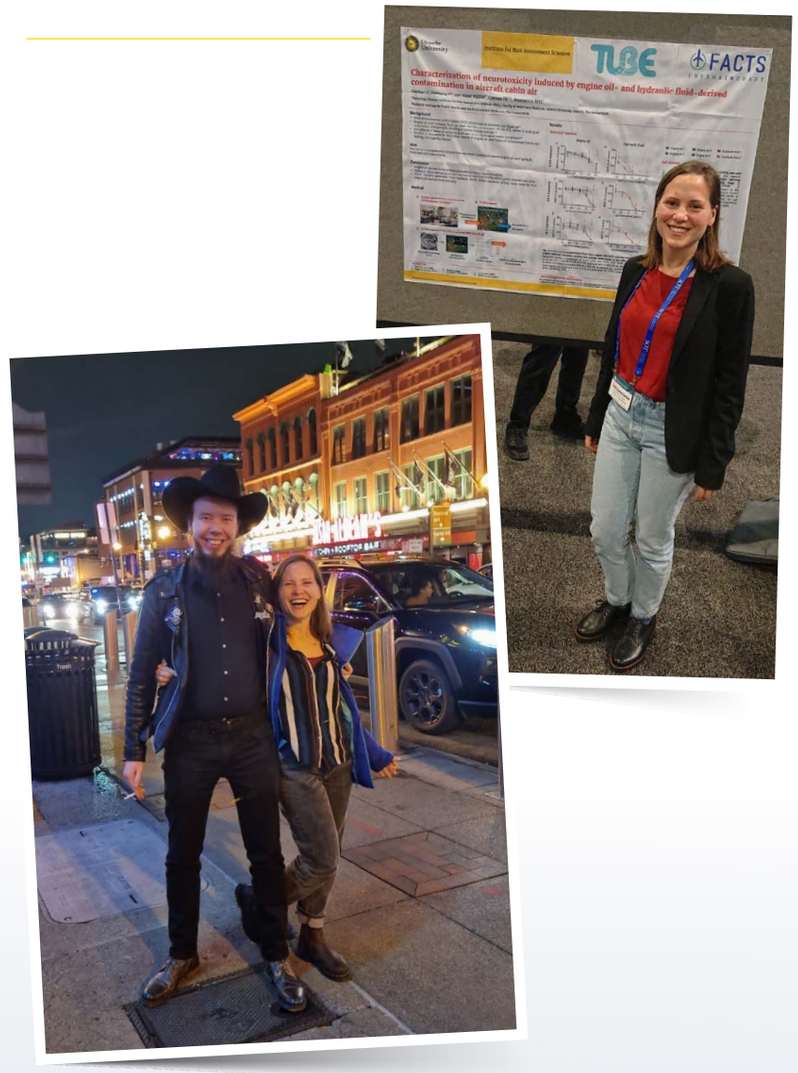
such fume contamination and, therefore, my research aimed to determine its neurotoxic hazard and compare the neurotoxic potency of fumes originating from different engine oils and hydraulic fluids. My research demonstrates that fumes generated from four engine oils and two hydraulic fluids reduced neuronal activity without affecting cell viability indicating that they exhibit specific neurotoxicity. Most importantly, fumes deriving from the hydraulic fluids were significantly more potent than the fumes generated from the engine oils suggesting hydraulic fluids contain hazardous compounds which are absent in engine oils. I was pleased to see so many visitors to my poster and enjoyed the great suggestions I received and the discussions I had.

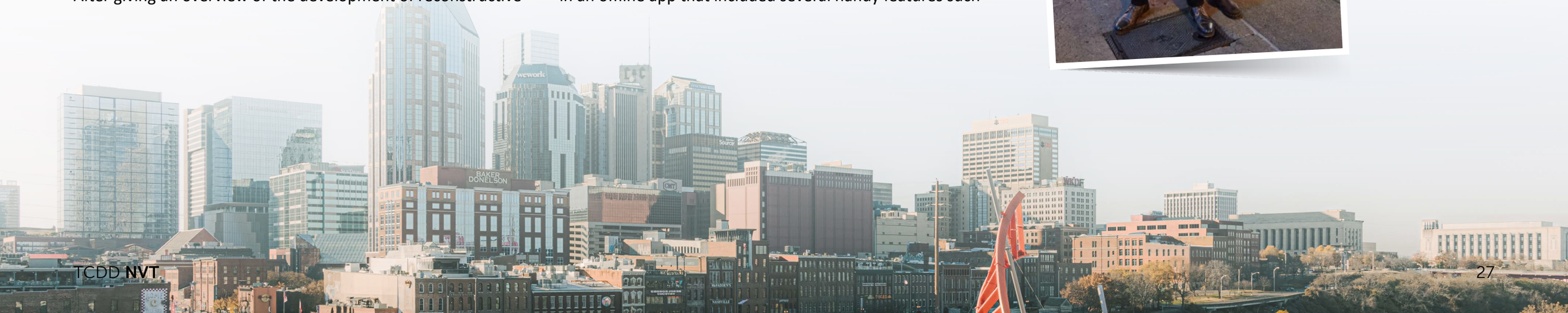
Besides this great experience, the greatest part about the SOT meeting was the variety of the program which covered all fields of toxicology making the SOT meeting an excellent platform to think outside the box and to see the bigger picture. I met many fellow researchers working on traffic-derived air pollution or neurotoxicity and enjoyed exchanging our knowledge and challenges.

Many talks were given to highlight the importance of further developing and validating new approach methods for risk assessment. One of my highlights was Dr. Helena Kadyrova's EUROTOX award lecture entitled "alternative methods and 3D tissue models: from initial idea to regulatory acceptance". After giving an overview of the development of reconstructive human 3D tissue models over the past 40 years she shared her experience working with human skin models. Dr. Helena Kadyrova pointed out the requirements and difficulties of implementing such models in regulatory guidelines and discussed the advantages and disadvantages of using commercial vs. "home-made" tissue models. Also, SynVivo organized a session presenting several organ-on-chip models including amongst others blood-brain barrier, lung, and inflammation on a chip. From these and many other talks, my main take home message was the fact that a deep understanding of the model and the relevant endpoints as well as applying the most appropriate exposure conditions are crucial for predictive toxicity testing.

Besides numerous great posters, I want to highlight Aiman Abzhanova's work. Aiman developed an impressive approach to study the oxidative effects of wood combustion emission in human airway epithelium. She uses primary human airway epithelial cells expressing the fluorogenic glutathione redox potential reporter Grx1-roGFP2, exposes them to woodsmoke generated in real-time, and assesses intracellular redox changes by live cell imaging. That way, she not only found that wood smoke does reduce time- and dose-dependently intracellular redox homeostasis, but also that effects were not attributed to the carbon monoxide in the mixture.

Worth mentioning, the SOT meeting program was available in an offline app that included several handy features such as pop-up reminders, discussion options, search options and uploaded e-posters making it easy to organize your day and to navigate at the venue. ⇨



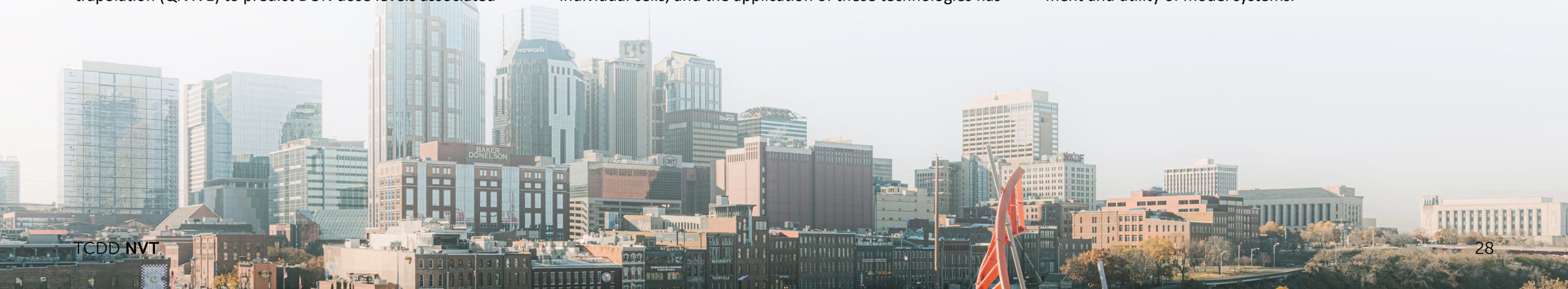


TRAVELER:
Jingxuan Wang,
Division of Toxicology,
Wageningen University
and Research

1. Describe what your presentation was about.
The presented poster focused on the use of physiologically based kinetic (PBK) modeling to predict the metabolism of deoxynivalenol (DON), and its role in intestinal inflammation and bile acid kinetics in humans. DON is a foodborne trichothecene mycotoxin that can contaminate cereal-based food. Current points of departure and derived health-based guidance values for DON are based on studies in laboratory animals. However, laboratory animals show notable differences in biokinetic activity and metabolite pattern of DON compared to humans. In this poster presentation, we adopted new approach methodologies (NAMs) to predict *in vivo* toxicokinetic profiles of DON and dose response curves for its effects on intestinal pro-inflammatory cytokine secretion and bile acid absorption in humans. Firstly, we established and validated PBK models for DON and glycochenodeoxycholic acid (GCDCA) homeostasis in humans. These PBK models were applied to perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) to predict DON dose levels associated with intestinal pro-inflammatory cytokine IL-1β secretion and GCDCA malabsorption. The results obtained were compared to one another and to the animal-derived point of departure and to human dietary DON exposure levels in various populations. This presented study provides a new testing strategy to investigate the effects of DON on bile acid homeostasis in humans.

2. Describe the three most interesting learning/insights you took from the conference. Summarize the most interesting other presentation(s) and describe a valuable new technique that was presented.
The recent development in single-cell technology has opened up new avenues for drug metabolism research. At the opening ceremony of SOT 2023, Dr. Namandje Bumpus of the FDA spoke about the use of single-cell technology to study the function of P450s, particularly the CYP2B6 enzyme and its interaction with efavirenz and its analogs. The presentation focused on the function of oxazinone ring of efavirenz, which was found to contribute to substrate specificity. Analogues were used to probe CYP2B6 activity, and these analogs were found to activate the 1RE1α signaling cascade, which is related to ER stress. The presentation discussed the use of matrix-assisted laser desorption/ionization (MALDI) coupled to mass spectrometry to study drug distribution in tissues, as well as single-cell proteomics workflows to study individual cell responses to treatment.
Recent advancements in single cell technologies have enabled detection of RNA, proteins, metabolites and xenobiotics in individual cells, and the application of these technologies has

the potential to transform research in toxicology. Single cell analyses can be utilized to expose underlying variability in cellular response to xenobiotics, providing a unique examination of efficacy, toxicity and metabolism. Proteomics performed at the level of individual cells can comprehensively characterize heterogeneity in cell-to-cell xenobiotic outcomes, including drug-induced toxicity, which enhances progress toward personalized and precision medicine. Further, understanding at the single cell level can facilitate more informed translation of preclinical data. This presentation discussed the use of single cell proteomics in understanding mechanisms of xenobiotic-induced toxicity and how insight into cellular heterogeneity might be incorporated preclinically to strengthen the development and utility of model systems. ⇨



3. Describe why a person (researcher) that you met during the conference really inspired you or could be useful for your further research.
One researcher who I found inspiring at the conference was Dr. Li from the University of Nevada Reno, along with his colleagues. Their work on modeling human intestinal absorption using the macroscopic mass balance approach (MMBA) caught my attention. Unlike the commonly used *in vitro* Caco2 cell model, MMBA is a flow model approach that utilizes fundamental mass transfer theory to estimate the extent of absorption for passively and non-passively absorbed drugs. Their approach can be applied to a wide range of chemicals, which could be useful for my own research.

4. Give your scientific "take home message" from this conference.
The use of novel approach methodologies, such as physiologically based kinetic (PBK) modeling, can provide valuable insights into the toxicological effects of compounds and aid in risk assessment.

5. Finally, describe in what ways the conference organization and yourself strived to make the conference a climate neutral event.
SOT have taken measures to reduce their environmental impact by promoting virtual attendance, reducing paper usage by developing the SOT event APP and encouraging sustainable transportation.

3Rs Student Grants 2023:
Call for submissions

The European Partnership for Alternative Approaches to Animal Testing (EPAA) supports students and young scientists with outstanding work in the field of alternative approaches for attending a high-profile scientific event.

Every year, a number of high-profile international meetings bring together world-class scientists working on the development and acceptance of 3R alternatives to animal testing (Replacement, Reduction or Refinement). Costs linked to participation may prevent students with promising work or young scientists at the beginning of their career from attending these events. The EPAA partners are therefore happy to sponsor the **3Rs Student grants** to facilitate the participation of students and young scientists in such events.

A jury will assess the applications and propose a list of selected candidates to the EPAA Steering Committee. The jury will be composed of 4 members (two from the Industry and two from the European Commission) who will judge.

In 2023, for the eligible events, 1 super grant of 2500€ for the 12th World Congress on Alternatives and Animal Use in Life Sciences and 2 full grants of 1000€ for EUROTOX 2023 are available.

- All grants cover the reimbursement of the event registration fees for the student/young scientist as well as travel and accommodation fees, on the basis of the expense receipts.

Eligibility

Eligibility criteria for both students and events are defined as follows. Applications falling out of this scope will not be accepted.

Students

1) Applicants have to be based (i.e. studying) in one of the 27 EU Member States or the UK. Citizenship may be from any country (i.e. also outside of the EU).
2) Applicants must be graduated (BSc / First cycle completed with at least 180 ECTS credits) when applying and should not have a doctoral degree.
3) Applicants must still be considered as full-time students or young scientists (≤ 35 years old) when applying.
4) Young scientists employed by industry are not eligible
5) No funding or reimbursement by another entity for the same student and event is allowed.

Events

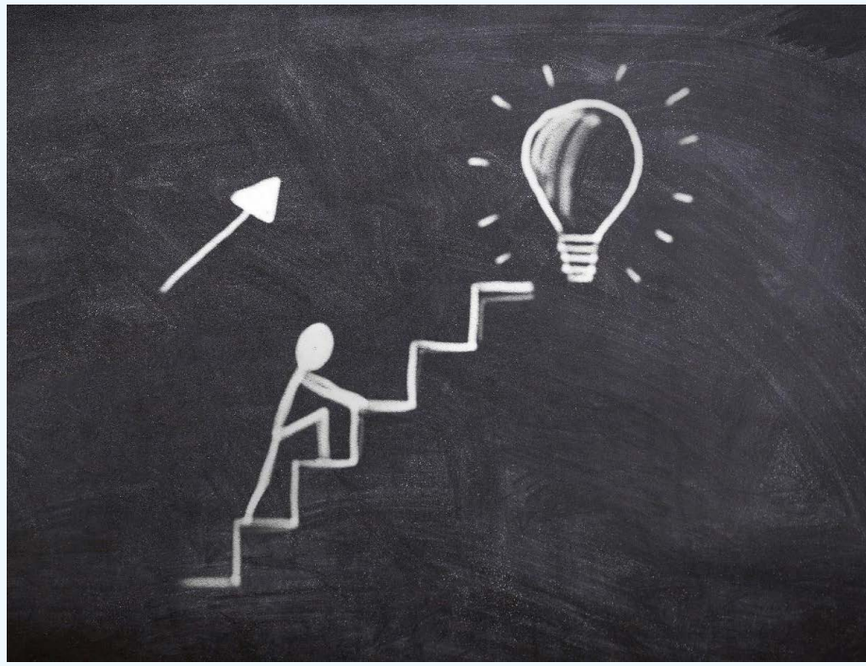
Selected high-profile scientific meetings are eligible for the EPAA 3Rs student grants. For 2023, the following events are open for application:

Event	Deadline for the application
12th World Congress on Alternatives and Animal Use in Life Sciences (Niagara Falls, Canada, 27-31 August 2023)	05/06/2023
EUROTOX 2023 (Ljubljana, Slovenia, 10-13 September 2023)	05/06/2023

Application and selection process

The application process is as follows:

1) Applications must be sent to EPAA functional mailbox (grow-epaa@ec.europa.eu) by midnight of the day indicated above
2) The Award will be granted provided an abstract is accepted for an oral presentation. At the sole discretion of the Jury, in exceptional cases work presented in a poster format will be eligible, too. EPAA visibility must be guaranteed by including a clear acknowledgment of EPAA's support in the presentation/poster. ⇨



Selection will be made by the jury on the basis of the following documents to be provided by the applicant:

- Abstract submitted for one of the selected events
- Detailed CV of the applicant with list of publications (if any)
- Cover letter describing why the work described in the abstract is important for the 3Rs and why it should be supported by the EPAA grant
- Recommendation letter from a professor/supervisor also confirming the applicant is a full-time student
- Proof of acceptance to the event (i.e. invitation to the event or confirmation mail)

Enquiries should be directed to grow-epaa@ec.europa.eu

EPAA recognition

The winner of the grant accepts to give recognition to EPAA in their presentation/poster. The winner is required to provide a proof which clearly shows him or her giving recognition to EPAA (a photo or a video with oral statement).

The winner of the grant also accepts that the EPAA may publish the abstracts of the presentation / the poster on its website (after the event), in order to promote and disseminate the research. A short summary report of the event and how the student benefitted from the participation needs to be provided.

Refinement Prize 2023

Call for submissions

The European Partnership for Alternative Approaches to Animal Testing (EPAA) is proud to announce its 2023 Refinement Prize. This prize of €6000 will be granted to a laboratory technician, animal care- taker or technologist who has demonstrated outstanding achievements in new, novel approaches to advance implementation and/or awareness raising of Refinement of animal testing.

PURPOSE

Laboratory technicians, technologists and animal caretakers carry out much of the work using animals for regulatory safety and efficacy testing purposes and are thus closely involved in efforts to apply Refinement strategies in such studies. Refinement is one of the 3Rs (Replacement, Reduction and Refinement of testing on animals). Refinement refers to the modification of any procedure, husbandry and care practices with laboratory animals along their entire lifetime, so as to minimise pain and distress and enhance their well-being.1

The purpose of this prize is to target those actually implementing alternative approaches to animal testing and/or raising awareness of their role for the day to day application and innovation of the Refinement principles in particular.

PRIZE

The EPAA partners will sponsor a 6000€ prize to be awarded in 2023. Applications can be submitted by an individual or on behalf of a team. The team must consist of at least one laboratory technician, technologist, or animal caretaker. The team can be supported by maximal one young scientist (master student, PhD-student or Postdoc). The money awarded is a prize, not a grant, therefore it does not require any prior justification of how it will be used. Nevertheless, the EPAA recommends linking its use to (further) promotion of alternatives, e.g. for travel and accommodation fees for a conference, publication costs, etc. The winner (or the ⇨

winning team) will be invited to the EPAA Annual Conference in November 2023 to receive the prize and briefly describe his/her/their contribution to the 3Rs.

EPAA will publish and/or communicate about the methodologies described in the winning submission, as well as those of runners-up at the discretion of the jury to facilitate widest dissemination and uptake.

ELIGIBILITY CRITERIA

Application is strictly restricted to candidates who:

1) Work in a laboratory and/or an animal facility as a technician, technologist or animal caretaker, master student, PhD-student or Postdoc
2) Contribute significantly to the development, wider implementation and/or promotion of a Refinement approach
3) Work in a European entity (EU Member States and neighbouring countries2).

[1] Definition of Refinement in for the purpose of this call as taken from: : http://ec.europa.eu/environment/chemicals/lab_animals/3r/alternative_en.htm

[2] Applicants from the following countries may apply: EU Member States, UK, Switzerland, Norway, Iceland, Monaco, Andorra, Liechtenstein, San Marino, Serbia, Bosnia and Herzegovina, Albania, Montenegro, Kosovo, Ukraine, Moldova and North Macedonia.

4) Agree to make themselves available for the EPAA Annual Conference on 15 November 2023

Please carefully note that any application not fulfilling all of the aforementioned eligibility criteria will not be considered. Applicants from previous winning teams may be eligible provided they base their new application on a completely different case study.

SELECTION CRITERIA

a) Impact on animal welfare – evidence based
b) Creativity and innovation
c) Practicability, applicability and implementation potential to regulatory testing and/or quality control
d) Potential for wider impact beyond immediate area
e) Publication of data or potential for publication

APPLICATION PROCESS

To apply, eligible candidates should send the following documents by email to: grow-epaa@ec.europa.eu before 12:00 CET on Monday, 18 September 2023:

- a cover letter (max. 500 words) explaining why the evaluation committee should consider the applicant's application;
- a brief CV, listing in particular relevant (refinement) work experience;
- a case study (max. 5 pages) describing the Refinement approach developed/applied/promoted and describing how the application meets the above selection criteria. Include pictures, video or other evidence as appropriate.

A selection panel comprising representatives of the EPAA partners (European Commission and industry) as well as of the EPAA Mirror Group will evaluate the submissions and propose a short list to the EPAA Steering Committee. The decision of the EPAA Steering Committee will be communicated to the applicants by 16 October 2023.The decision is always final.

TIMELINES

Indicative timelines are as follows:

- 8 March 2023: Launch of the call for applications.
- 18 September 2023: Deadline for submission of applications.
- 16 October 2023: Information of Applicants on the outcome of the selection procedure.
- 15 November 2023: Winner to speak at the EPAA Annual Conference.

ABOUT EPAA

EPAA is a Public-Private Partnership across seven industry sectors and between European Commission and Industry stakeholders. Launched in 2005, it gathers 38 companies, 8 European trade federations and 5 Directorates-General of the European Commission.

Further information is available on:
https://single-market-economy.ec.europa.eu/sectors/chemicals/european-partnership- alternative-approaches-animal-testing_en
EPAA Annual report 2022:
https://single-market-economy.ec.europa.eu/document/02716de3-deed-4a38-9b09- 75f7df3f4922_en

Puzzle TCDD 2023-1 solution

H	U	R	R	A	D	E	V	E	L	O	P	M	E	N	T	A	L	Y	Y	O	U	C	I	D
R	L	A	I	C	I	F	I	T	R	A	N	I	M	A	L	I	D	K	I	T	Y	O	N	O
U	E	M	L	A	C	I	T	U	E	C	A	M	R	A	H	P	A	O	N	A	G	M	T	E
S	A	F	E	T	Y	D	V	A	C	C	I	N	E	T	O	F	I	R	N	D	B	P	E	A
L	L	T	L	Y	G	E	T	A	R	T	S	C	O	V	I	D	H	R	E		O	U	L	O
R	V	G	D	E	S	I	N	O	V	U	P	R	P	I	O	U	Z	E	Z	L	R	T	L	E
H	I	O	R		C	E	V	E	E	D	O	B	U	I	T	E	N	L	A	N	D	A	I	R
R	R	Y	O	E	U	T	A	R	I	E	R	N	O	T	D	A	D	S	O	N	E	T	G	E
F	U	T	U	R	E	T	I	H	L	O	T	U	U	G	H	Y	N	S	O	U	R	I	E	A
M	S	B	F	R	E	N	N	N	I	E	S	A	G	R	L	Y	T	R	E	H	S	O	N	E
I	R	R	B	R	E	H	O		G	E	N	E	E	S	M	I	D	D	E	L	E	N	C	L
C	O	E	F	E	E	T	E	C	H	E	M	I	C	A	L	S	N	D	I	T	D	A	E	I
R		X	A	G	R	E	N	Z	E	N	L	O	Z	E	E	U	S	E	T	T	N	L	L	F
O	H	I	L	F	E		O	R	I	D	T	O	T	O	X	I	C	O	L	O	G	I	E	E
P	X	T	T	O	S	T	C	D	D	I	N	N	O	V	A	T	I	V	E	X	I	N	B	S
L	C	O	E	R	L	C	O	G	Y	I	N	O	X	U	R	T	C	H	E	I	M	H	R	T
A	E	E	R	E	F	C	H	D	I	T	O	X	I	C	T	M	R	I	O	C	N	A	A	Y
S		S	N	V	F	O	R	E	N	S	I	S	C	H	E	I	I	T	H	O	I	L	T	L
T	S	A	A	E	A	R	O	M	I	O	U	N	I	F	T	X	S	I	S	L	T	A	I	E
I	H	E	T	R	R	O	E	D	A	D	L	A	T	E	S	T	I	N	G	O	N	T	O	S
C	S		I	E	E	N	R	T	O	D	N	O	Y	E	B	U	S	T	H	G	E	I	N	P
S	U	Z	V	Z	R	A	L	E	P	L	E	A	S	D	E	R	S	E	N	Y	D	O	S	T
E	X	P	E	R	I	M	E	N	T	S	H	I	S	N	U	E	M	B	E	R	T	N	O	O
U	R	E	S	M	A	E	C	O	T	O	X	I	C	O	L	O	G	Y	I	L	A	S	L	I
S	T	E	D	A	B	O	P	E	T	R	O	L	E	U	M	V	E	O	R	B	E	L	O	

Instructions in TCDD 2023-1: Cross out all the bold words from the list of theme topics. Use the remaining letters to find the puzzle's solution (extra hint: you can place the letters in chronological order on the dots below). Send your solution to redactie@toxicologie.nl and you can win a prize!

Hurray, you did it! You managed to find all the words in our puzzle. However, you are not done, though you are nearly there. **How often did we use the word toxicology in our theme editions?** This amount is the real answer to the puzzle please send this number to our email as listed above.

TERUG NAAR INHOUD