# TCDD

TOXICOLOGIE

NUMMER 3
OKTOBER 2020


SPECIAL THEME

# ECOTOXICOLOGY 2020

- MAURITIUS OIL SPILL SUMMER 2020
- THE IMPACT OF THE FIRE OF NOTRE DAME DE PARIS' ROOF ON THE ENVIRONMENT
- A BIRD'S-EYE VIEW, A WILDLIFE PERSPECTIVE ON ENVIRONMENTAL RISK ASSESSMENT OF CHEMICALS
- ECETOC DEVELOPS CF4POLYMERS

NEDERLANDSE VERENIGING VOOR TOXICOLOGIE

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**Toxicologische Communicatie, Data en Documentatie**

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TCDD
TOXICOLOGIE

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TERUG NAAR INHOUD

# Editorial

"Het zijn vreemde tijden, hele vreemde tijden. Een wereldwijde pandemie houdt ons allen in de greep". Dat waren de eerste zinnen van mijn collega-redactielid Martje de Groot in de vorige TCDD. Ik had gehoopt, misschien tegen beter weten in, dat haar woorden niet meer van toepassing zouden zijn bij het verschijnen van deze editie, maar helaas zitten we er nog midden in. "Het Coronavirus blijft voorlopig onder ons" is wat onze minister president vertelde tijdens zijn toespraak op 16 maart dit jaar. Nu weten we dat die voorspelling juist is geweest. Hopelijk hebt u allen inmiddels een modus gevonden om met de huidige situatie om te gaan, zoals een nieuwe (thuis)werk-privé balans en een fijne manier om in contact te blijven met familie, vrienden en collega's.

Gelukkig is de situatie niet meer exact hetzelfde als bij het verschijnen van de vorige TCDD. Inmiddels zijn vele malen minder IC bedden bezet, zijn er een aantal maatregelen versoepelt en heeft de ontwikkeling van een vaccin flink progressie gemaakt. Laten we hopen dat deze positieve ontwikkelingen zich blijven voorzetten en dat onze hoofdredacteur Barae Jomaa in de volgende editie meer goed nieuws kan vermelden in het redactioneel voorwoord.

Om vanaf nu te focussen op de huidige TCDD die voor u ligt: het thema van dit nummer is Ecotoxicologie. In het kader van dit thema hebben we voor u een aantal actuele artikelen over onderwerpen waarbij ecotoxicologie prominent in het nieuws is geweest. Zoals de impact van de brand in de Notre-Dame in Parijs, het voorstel van het Europees Chemisch Agentschap (ECHA) voor een EU-wijd verbod voor bepaalde microplastics en de gevolgen van de olieramp bij Mauritius deze zomer. Daarnaast vindt u in deze editie een themastuk over de toenemende vraag naar geschikte neurotoxiciteit-eindpunten bij de (eco)toxicologische evaluatie van onze waterkwaliteit. Onze vaste rubriek "Toxafette" sluit dit keer toevalligerwijs ook aan bij ons thema; Laro-Sophie Gerber vertelt ons alles over haar promotieonderzoek naar het neurotoxicologisch potentieel van verkeersgerelateerde ultrafijn stof.

Uiteraard zijn ook de andere, niet aan het thema gerelateerde artikelen zeer de moeite waard om te lezen. Echter, tot slot wil ik graag nog één stuk voor u uitlichten: het in memoriam aan Henk Tennekes. Hij is overleden op 7 juli jongstleden en met zijn overlijden is een gewaardeerd lid van de toxicologische gemeenschap weggevallen. Als toxicoloog was hij het meest bekend vanwege zijn invloedrijke onderzoek naar neonicotinoïden. Op pagina 21 vind u een samenvatting van de gedenkwaardige aspecten van zijn (professionele) leven.

Namens de redactie,

Maaike Steenhof

Photo by Jess Bailey on Unsplash

# **News** from the board

Welcome to the October 2020 issue of TCDD, which is dedicated to a field of toxicology that is close to my heart: ecotox!  I'm sure you are looking forward as much as I am to this exciting issue created by our terrific TCDD editorial team with input from our members.

Looking back on the past few months, what a strange time it has been, with workplaces slowly opening up again after the lockdown, uncertainties about the future, and worries about a 2nd wave. From the NVT Board perspective, this period marked the first time ever that the Annual Meeting/PhD days were postponed, and that the Member Assembly ('ledenvergadering') meeting was held online through MS Teams! Though I missed not being able to interact with members in person, it was great that over 80 people attended the online meeting. Many thanks to those who attended.  Keep an eye on announcements in the coming period for the date and location of the 2021 NVT Annual Meeting/PhD days.

A few highlights from the Member Assembly meeting of June 11, 2020 (which are described in more detail in the minutes of the meeting):
Looking back on 2019, we granted a record number of 16 travel bursaries to PhD candidates. New software for re-registration was finalized and went live. We signed a Memorandum of Understanding with IUTOX and EUROTOX at ICT 2019. The NVT board nominated a number of candidates for different positions and awards that were successful: Henk van Loveren was chosen as board member of IUTOX and Theo de Kok as board member for EUROTOX. Martin van den Berg was accepted as member of the education committee of EUROTOX, and at the 2019 Eurotox meeting, the Bo Holmstedt Award was awarded to Wout Slob. In 2019, we also installed a new president of the Board (Juliette Legler) and a new secretary of the NVT (Nicole Nijhuis). At the end of 2019, our Society totaled 631 members. Financially, we just about broke even in 2019, and maintain a healthy reserve. The registration committee for toxicologists reported that in 2019 there were 73 Toxicologist-in-Training and 309 registered toxicologists. Regarding the Postgraduate Education in Toxicology, three new courses will be offered (Epidemiology, Occupational Toxicology  & Exposure Assessment, and Legal and Regulatory Toxicology) and much effort is ongoing towards developing online modules for existing courses.

At the meeting, we also presented the Joep van de Bercken prize of 2020 to Carin Lunenburg for her thesis "Personalised medicine of fluoropyrimidines using DPYD pharmacogenetics". Carin's thesis was selected by a committee of  5 experts from academia, private and public organisations, who were impressed by her discovery and clear description of diagnostic markers that may aid in the mitigation of very severe side effects of specific cancer drugs. Carin has kindly agreed to present her PhD work at the annual meeting next year; we are looking forward to congratulate her and hear more about her work in person!  Congratulations Carin!

Finally, the Member Assembly ended by wishing three of our Board members farewell, and welcoming three new Board members.  Many thanks to our former (vice-)president Henk van Loveren, secretary of the Board Minne Heringa, and Board member Rob Stierum for their dedication over the past years. And a warm welcome to our newly installed members Vice-President Prof. Paul Jennings, secretary of the Board Dr. Hester Hendriks, and Board Member Dr Anne Kienhuis.
Thank you for taking on these roles, and we look forward to working with you!

All the best and take care!

**Juliette Legler**
*President NVT*

SECTIE RISICOBEOORDELING

# ONLINE Autumn symposium: ‘Everything you wanted to know about PFAS’

## Date: November 5th, 2020 (13.00 – 16.30 h)

Location: ONLINE

| Voorlopig programma: | |
|---|---|
| **13.00-13.15** | Registration |
| **13.15-13.30** | Welcome & instruction |
| **13.30-13.50** | Introduction – Prof. dr. Annemarie van Wezel (UVA) |
| **13.50-14.20** | Determination of PFAS limits in soil – Dr. Arjen Wintersen & Dr. Piet Otte (RIVM) |
| **14.20-14.50** | Policy consequences – Dr. Marije Schouwstra (I&W) |
| **14.50-15.00** | Coffee break |
| **15.00-15.20** | From soil to (drinking) water – Dr. Frederic Béen (KWR) |
| **15.20-15.40** | From water to livestock to consumers – Dr. Krista Bouma & Dr. Jacqueline Steenbergen – Biesterbos (NVWA) |
| **15.40-16.30** | Discussion & wrap-up |


Source: NOS

You can register for this meeting by sending an email to Monique Nagtegaal (m.nagtegaal@nvwa.nl). Please report “Registration NVT autumn symposium – 5 November” in the title of the email and report your name and affiliation in the email itself.

SECTIE MILIEUTOXICOLOGIE /
ENVIRONMENTAL TOXICOLOGY SECTION

## Annual symposium

# “How environmental chemistry, toxicology and geochemistry can be applied to solve environmental problems”.

We are happy to announce our annual symposium: “How environmental chemistry, toxicology and geochemistry can be applied to solve environmental problems”.

Date: **Thursday, January 21, 2021**.

The meeting is planned **online**, with all speakers at the same location to facilitate discussion (all pending regulations). At the symposium, the bi-annual Thesis award of the MCT-section will be presented.

SECTIE GENEESMIDDELENTOXICOLOGIE

## Update FIGON:

# Dutch Medicines Days (DMD)

Er zal geen fysieke FIGON-DMD in de Leidse stadsgehoorzaal plaatsvinden. In plaats daarvan zullen er in het najaar (tussen september 2020 en januari 2021) een aantal webinars georganiseerd worden, waarin de activiteiten/ voordrachten plaats zullen vinden die we normaal tijdens de FIGON-DMD doen.

Zo zal er een PhD competitie zijn, gethematiseerde sessies (zoals Discovery, Development en Use), key note lectures en gemodereerde abstractsessies. Deelname aan deze webinars zal kosteloos zijn.

Op 15 september vond de eerste ‘kick-off’ meeting plaats (opening + key note lecture). Dit gebeurde in de ochtend voorafgaand aan bovengenoemde abstractsessies.

Abstract submissie voor de FIGON-DMD is mogelijk op de website van de FIGON-DMD. Wij willen de leden vragen om hun wetenschappelijk werk als het even kan te submitten.

De FIGON blijft een essentiële organisatie voor onze vereniging. Binnen de FIGON vinden een paar belangrijke ontwikkelingen plaats, zowel op het gebied van onderzoek als onderwijs, waar de organisaties die zich in Nederland bezighouden met geneesmiddelenonderzoek in de volle breedte van kunnen profiteren. We willen u daarom ook vragen om vooral deel te nemen aan de webinars die in het najaar georganiseerd zullen worden.

# The Impact of the Fire of Notre Dame de Paris' Roof on the Environment

*By Héloise Proquin*

**The date stays in the hearts of the French population but also in the hearts of many from all around the world: 15 April 2019. Many people, including myself, looked at this majestic monument as it burst into flames. Not only did it have an impact on many people but it also had an impact on public health and the environment.**

The roof of Notre Dame de Paris was made of two main materials: wood, which they called the forest because it literally took timber from an entire forest to build the framework of which the impact is too difficult to assess 700 years later, and lead. It took 210 tons of 5 mm tick lead to build the roof plus 250 tons to build the spire of the cathedral. This makes a total of 460 tons. The melting point of lead is 327°C and the estimation of the temperature of the heart of the fire was between 600°C and 900°C. At this temperature, lead is in fusion and can be vaporised. Its dissemination in the atmosphere in the form of microparticles is a hazard to public health and the environment. Lead sticks to dust and elevated concentrations of lead in the air have been measured up to 40 km away from the cathedral in Limay (Yvelines)[1].

Image: Guillaume Levrier

Photograph of Notre Dame's spire taken from the Saint Louis Bridge during the 15th April 2019 fire

In order to assess the potential impact of lead release in the environment, it is essential to refer to threshold limits which evaluate the potential danger in: air, inside the building, water, and on the ground. These limits have been well assessed except for the latter, which had no regulation before the fire. Indeed, limits on the ground have never been assessed and a reference concentration had to be estimated in order to differentiate the background noise coming from the daily pollution of lead in Paris (many of the old pipes are made of lead as well as many old roofs and paints) from the pollution due to the fire. The regional health agency (ARS) therefore decided that the normal concentration of lead on the ground of Paris was 5 000 µg/m$^2$. They based this concentration on two studies performed by the regional head of cultural affairs (DRAC) during the follow up of historical monuments. Scientists agree that none of these studies show results from which a threshold could be deduced[2]. When the responsible persons of these studies were contacted, they have said that these studies were not performed to answer questions regarding public health[2]. Additionally, based on the results of one of these studies, an average was calculated at 1346 µg/m$^2$ and only two measurements were above 5 000 µg/cm$^2$ [3]. The scientific validity of this threshold, as well as the impact ➜

evaluated from this new regulated concentration, can be questioned.

In order to assess the environmental impact, one would then conclude that it would be simple to measure the lead concentration in the Seine which can be compared to already assessed regulatory limits. However, no studies have been performed to assess the impact of the fire on the biodiversity in the Seine.

This unfortunate event shed light on the fact that not all types of environmental pollution have been assessed. More studies are needed to be able to estimate different impacts of the Notre Dame fire on nature. Toxicology still has a bright future. ■

**References:**

1. AirParif, https://www.airparif.asso.fr/actualite/detail/id/267
2. Le Monde, 29 July 2019, https://www.lemonde.fr/les-decodeurs/article/2019/07/29/incendie-de-notre-dame-le-seuil-de-concentration-du-plomb-choisi-par-les-autorites-est-il-dangereux-pour-la-population_5494674_4355770.html
3. Concentrations of lead in the air from DRAC, 2017; https://assets-decodeurs.lemonde.fr/decodeurs/medias/notre-dame_plomb/DosagePlomblingettes.xls

Photo by Nivenn Lanos on Unsplash

# Mauritius oil spill summer 2020

*By Tinka Murk, Marine Animal Ecology group Wageningen*

On July 25, 2020, the Japanese but Panama-flagged cargo ship MV Wakashio ran aground near Blue Bay Marine Park of the Indian Ocean paradise island of Mauritius. The Wakashio belongs in the top 1% of largest ships in the world and is too large to fit through the Suez Canal, and hence traveled around the coast of Mauritius from China to Brazil. The single-hull bulk carrier transported 4,000 tons of oil and diesel of which at least 1,000 tons leaked from a gaping crack over pristine coral reefs, internationally protected turtle habitats and core habitats of endangered marine mammals. Three weeks later, on the 15th of August, the vessel split in two and on the 24th of August half the vessel was deliberately sunk at a still undisclosed location. Local media in Mauritius reported, on 18 August, 2020, that the broken front half of the vessel would be towed 8 miles to the East of the island to sink it, a famous nursing ground area for whales and their calves.

Floating spilled oil is especially threatening to air-breathing marine animals, such as sea turtles, marine mammals, as well as sea birds that land in the oil and get smothered. Floating oil can also pollute mangrove forests, swamps and beaches for decades to come. Therefore, oil spill responders try to bring the oil down from the surface. Relatively soon after the spill started, 49 dead whales and dolphins washed ashore, which was more 'acute' than usual. In previous oil spills, such as the Deep Water Horizon in the Gulf of Mexico, oil-exposed marine mammals developed a respiratory illness that killed many of them. In the case of the Mauritius spill, it seems that the oil spill response contributed to the mortality. When dispersants are being applied to break the oil up into small droplets, especially the more hydrophilic toxic compounds are all released at once. This should only be done in deeper water (ideally more than 60m deep) to ensure fast dilution to concentrations below those inducing acute effects. Therefore, before any spill occurs responsible governments should decide when and where dispersants can be safely applied, or what else to do. In addition, the effects should be tested under realistic local environmental conditions. During the Deep Water Horizon blowout, application of dispersants during an algal bloom unexpectedly triggered marine snow formation that subsequently collected dispersed oil and particulate matter, resulting in a so-called 'dirty blizzard' that concentrated the oil at the deep sea floor instead of diluting it and made it more persistent instead of enhancing biodegradation. So dispersant application could smother shallow coral reefs as well as real deep sea benthic ecosystems, and the impact can last for decades.

I tried to find more information about the fate and effects of the spilled oil, and what struck me is the lack of good information. The entire South Eastern coast has been closed and international scientists seem not welcome to take oil samples for fingerprinting the type of oil, no animal samples are being taken for biomarker research and also the local people are not involved in the decision making.

The Government and the oil spill response team seem to be quite disorganized, although they recovered quite some oil from the stranded Wakashio. Photo impressions of the Mauritius oil spill show that the oil reached the network of highly protected nature reserves including the pristine ➜

Wreck of the MV Wakashio pictured on August 17, 2020

barrier coral reef, mangrove forests, seagrass fields and stone beaches – in addition to smothering some of the most important sites of historical and cultural importance to Mauritius. Affected historical istes include Dutch landing where Dutch explorers landed on the Island of Mauritius in 1598 and on a sketch you can see Dutch sailors exploring, hunting and taking a turtle-back ride. Mauritian citizens started to make homemade oil protection booms from dried sugar cane leaves, plastic bottles to keep these afloat, items of clothing and human hair in desperate struggle to get rid of the oil.

Oils spills and almost-disasters from oil transport occur on a too regular basis. For example, the Indian Ocean was on full alert again when an oil supertanker caught fire off the coast of Sri Lanka and began spilling oil on the 3rd of September. This tanker, the MT New Diamond, is a Very Large Crude Carrier and carried twice the amount of oil of the Wakashio. In the Red Sea an abandoned massive Yemeni oil tanker is threatening the entire Red Sea region as it can explode any moment and sink. In the meantime Venezuela experiences a large oil spill in its national park since the 2nd of August. It is truly shocking how often aged and/or single-hulled oil tankers can so easily threaten the precious last rich marine ecosystems. There is insufficient local and international regulation and preparedness to deal with massive spills, and local people can, in a few days, lose their livelihood as seafood stocks can be wiped out or made unsafe to eat. Needless to say, tourists stay away for many years after a spill occurs. It is important to have internationally-agreed and organized shipping zones, contingency plans, pre-impact monitoring and also involvement of local stakeholders.

IMO workers in hazmat suits stand in surf near the wreck on August 13, 2020

Still, my biggest worry goes further than these huge tankers containing thousands of tons of oil. Massive risks are in the make from oil drilling and production in the vicinity of key coral and marine wildlife areas along the African coasts. For example two major oil and gas projects, under development on the border with Senegal and Mauritania, are expected to start producing in 2022/23 and an oil and gas project in the Saloum Delta is expected to start production from 2023/24 onwards. These areas are a key habitat for hundreds of by-now rare species including the largest global colony of the Royal tern and the last viable population of Atlantic humpback dolphins in West-Africa. The same developments occur along the East African coast. An oil well blowout can potentially result in even more massive oil contamination than super tankers can spill. Also the oil needs to be transported via pipes and tankers that can leak and break. It is not a question whether, but when the first environmental disaster will take place and also in that case, as with Mauritius, local authorities cannot be expected to handle the situation without international support. And, in addition to the discussed consequences for the marine environment, also then local people will benefit least and suffer the greatest consequences for their economy, food security and health. The sooner we can cure ourselves from our fossil fuel addiction the better! ■


Satellite view of MV Wakashio oil spill and surrounding area (11 August 2020)

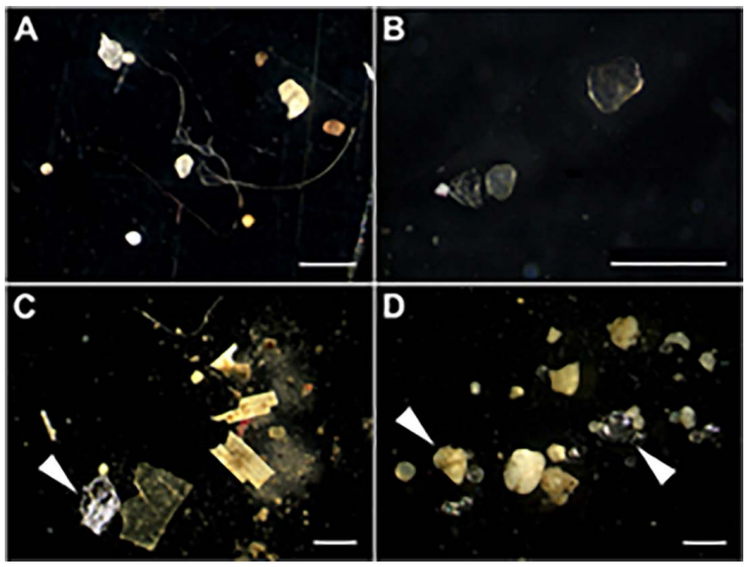
## European Restriction proposal on intentionally added microplastics

# Are public consultations really public?

*By Jasper Woutersen*

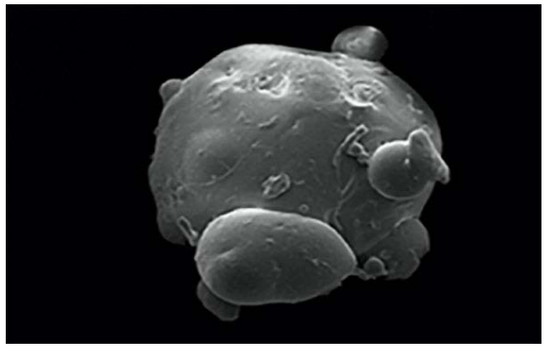
**In January 2019, the European Chemicals Agency (ECHA) published a proposed wide-ranging restriction on intentionally added microplastics, which describes a possible restriction on the use of persistent microplastics in products such as cosmetics, controlled-release fertilizers, detergents, and capsule suspended plant protection products and biocides. Ok, this is interesting, but maybe a refresher on the microplastic topic can come in handy before I continue explaining the issue here.**

So, the ECHA defines Microplastics as follows: "Microplastics are very small (typically smaller than 5mm) solid particles composed of mixtures of polymers (the primary components of plastics) and functional additives. They may also contain residual impurities from when they were manufactured. They can be unintentionally formed through the wear and tear of larger pieces of plastic, including synthetic textiles. They can also be deliberately manufactured and intentionally added to products for a specific purpose, for example, as exfoliating beads in facial or body scrubs." Ok, seems pretty logical, but now focus on the part between the brackets, **typically** smaller than 5 mm ...., that is not a very rigid and clear definition. Some articles or reports define microplastics as particles in the size range of 1 nm to < 5 mm, however you could also subclassify as, nano-, micro-, meso-, macroplastics. Although, most of the articles will refer to microplastics as being smaller than 5 mm, this still implies a very broad range of possible forms and sizes. Another side note I would like to add, before I continue, is that the microplastics that can be present in for example facial scrubs, that are flushed down the drain, also end up in the environment in a less expected way. In the beginning of the research for this article I wasn't aware that, after the filtration of the sewage the resulting sludge is being amended to our agricultural soil, coming therefore into our environment and in contact with the food we are growing.




Microplastics in sediment from rivers. By Martin Wagner et al. - Wagner et al.: Microplastics in freshwater ecosystems: what we know and what we need to know. In: Environmental Sciences Europe. 26, 2014, doi:10.1186/s12302-014-0012-7, CC BY 4.0, https://commons.wikimedia.org/w/index.php?curid=39507778

After this refresher lets continue with the story; the ECHA estimates the total environmental release of intentionally-added microplastics at approximately 42,000 tonnes per year in the European Union alone. To put this in perspective: it is also estimated that 41,000 tonnes of plastic pre-production pellets are lost to the surface water each year. The proposed restriction comprises of three different measures, the first measure is the restriction of placing microplastics on their own or in mixtures on the market. This restriction applies to all uses that inevitably result in environmental release, however there is an exception for microplastics that are biodegradable. The second measure, is the requirement for labelling from 2021 onwards of products that contain intentionally-added microplastics, like construction products, medical products and devices, food supplements, paint etc., where the microplastics are not inevitably released into the environment but could, when not used or disposed of properly. The final measure is a reporting requirement for all downstream users of microplastics or users placing product containing microplastics on the market. This to better monitor the effectiveness of the restriction and evaluate the need for further action. The restriction should be in place in 2021 with an appropriate transition time tailored to specific industries and after a public consultation. ➜



A microbead imaged using a scanning electron microscope. By University of Exeter from United Kingdom - Andrew Watts Face to Face with Plastic, CC BY 2.0, https://commons.wikimedia.org/w/index.php?curid=50504675

Public consultation is generally performed before a proposal is finalized as one of the cornerstones of the EU's governance-based policy strategy. These consultations are intended to increase the efficiency and transparency of regulations. All comments are therefore always published at the end of a consultation. The philosophy behind the public consultation is that successful involvement of stakeholders will increase the likelihood of smooth and long-term implementation of the regulatory actions. However, the question is, are the public consultations sufficiently able to reach all relevant stakeholders that the ECHA intends to reach? Clausen, Hansen, Oturai, Syberg, and Hansen (2020) performed a stakeholder analysis based on the abovementioned restriction proposal and this gives some interesting insides into the world of public consultation. The following part of the story is based on the article from Clausen et al. (2020): "The general result of the public consultation shows that stakeholders provide arguments mainly based on their own set of criteria. The stakeholder arguments can be divided into two different sides of a spectrum, one side includes arguments from stakeholders that are clearly sceptical, to say the least, and the other side includes arguments from stakeholders that are apparently happy with the restriction proposal. For example, industry and associated trade associations often provide arguments showing anxiety to the cost and the bureaucratic burden of mandatory labelling and the overall societal benefits of the restriction proposal. Whereas international NGOs and environmental NGOs show positive attitudes towards the upcoming restriction and welcome the ban on intentionally added microplastics. They also call for more research into the biodegradation of plastics, as these are exempted from the ban, opening a possibility for the search for alternatives. Large companies were the most active in giving comments during the public consultation, whereas international authorities gave very few comments. The latter stakeholder showed increased expression of their opinion in the media, whereas large companies did not speak out in the media. It is interesting to zoom in on the lack of involvement from certain groups of stakeholders. Small and medium business involvement was fairly low (four out of the 205 identified stakeholders), although the upcoming ban could have huge implications for these companies. In 2015, over 99% of the businesses, equivalent to around 23.4 million companies, were small and medium-sized. Academics and researchers gave no comments during the public consultation. Although some scientific feedback is provided by two committees, the Committee for Risk Assessment (RAC) has no plastic expert and the Committee for Socio-Economic Analysis (SEAC) has only one. This could result in a lack of balanced scientific background for the upcoming ban, which could undermine the scientific advice given by ECHA. And although the consultation is called 'public', the public is only indirectly represented through politics and NGO's. The public will however be affected by the restriction proposal as it could implicate that certain consumer products will no longer be available."

The last topic I would like to draw your attention to is the well-known phenomenon of stakeholders forming 'advocacy coalitions' to have greater influence on the policy making process. In the article by Clausen et al. (2020), the researchers suggest that the sports NGOs, which have interest in the current restriction due to the use of artificial turf on their sports fields, and the industry-related stakeholders seem to have formed an 'advocacy coalition' opposing the restriction. The two stakeholder groups seem to provide identical arguments and support the claim that the restriction would have severe negative social and economic effects. It is suggested by the authors that forming such an advocacy coalition, with the use of identical comments by the NGOs and heightened interest from industry-related stakeholders, might be an indication for an attempt to overburden the consultation process and slow down the restriction process.

You are probably thinking at this point, ok this is all very interesting but why should I care? Well, there are a couple of points I am trying to make here. First off all, although these are called public consultations, how well is the public represented and isn't there a big overrepresentation from the small amount of big companies? Do public consultations result in a false feeling of proper engagement with the public and a failure to consult the quiet or silent stakeholders? And secondly, as a scientific community we need to be more directly involved in these public consultations, not solely outing our concerns through the media. The suggested solution by inviting 3-5 guest experts with specific knowledge to the committees would be a great step in the right direction. ■

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# A bird's-eye view, a wildlife perspective on environmental risk assessment of chemicals

**Historically, wildlife has played an eminent role in the regulation of chemicals. The ban and use restrictions for most legacy POPs like PCBs and DDT were initiated by the adverse effects these chemicals had on, for instance, birds of prey, orcas, polar bears and other wildlife species. Since then, prospective assessment of the environmental risks of chemicals has evolved to meet higher standards. Elaborate tiered approaches have been developed and validated, enabling science-based, efficient risk assessment. Nevertheless, several cases have shown that adverse impacts may still occur, even when chemicals are used according to regulation.**

*By Nico van den Brink, Wageningen University*

For instance, in the early 2000s millions of vultures died in India, Pakistan and Bangladesh due to the use of diclofenac as a veterinarian drug, according to the label, nearly making these species going extinct. These vulture species appeared to be very sensitive[1], which was not picked up in regular risk assessment. Neonicotinoid insecticides are another group of chemicals which, although strictly regulated, may cause adverse effects in birds. These pesticides have shown to affect the ability of migratory birds to acquire the essential fat stores and also their navigation, when initiating migration[2]. The use of anticoagulant rodenticides to control rats and mice is strictly regulated. However, numerous studies have shown large scale effects on birds of prey and mammalian predators, due to secondary poisoning[3]. In our own ongoing research we have shown adverse effects of e.g. mercury on neurotransmitter levels and behaviour of Arctic Barnacle goslings at very low concentrations [4, 5], as well as immunomodulatory effects of trace metals on wild living small mammals and waterfowl, both known reservoirs of zoonotic diseases of e.g. respectively Lyme diseases and Avian influenza.

Picture of a 'charismatic' wildlife species (image credit: Nico van den Brink)

These issues, and others, indicate that current environmental risk assessment (ERA) is not completely 'waterproof' in preventing risks for wildlife. This may be due to the fact that species are more sensitive than expected (e.g. vulture case), but also because affected toxic endpoints are generally not considered in current risk assessment, such as, for instance, behavioural and immunomodulatory effects. Such effects may have serious impacts on the fitness of the animals involved, while chemical-induced immunomodulation in animals may also affect human health. From a wildlife perspective it is therefore extremely relevant to go beyond the current paradigms of environmental risk assessment, which mainly focus on impacts on populations via growth, reproduction and mortality of organisms. Including this in current testing schemes for prospective risk assessment frameworks may be complex and ➜

also ethically unwanted due to the associated increase in the use of test animals. However, I think this demands for an increased focus on risks of chemicals after they enter the market and are being used (posterior), with emphasis on toxic endpoints not addressed in regulatory risk assessment, e.g. behaviour, immunomodulation, genotoxicity. Not all chemicals can, and probably need to be addressed. Information from, for instance, human risk assessment may be used to select those chemicals that may potentially pose risks to wildlife under environmental long-term exposure scenarios. The assessment of the need of such posterior risk assessment can be part of the regulatory dossier that needs to be submitted. Monitoring of these chemicals may be based on animals found dead, see the Predatory Bird Monitoring Scheme (see: https://pbms.ceh.ac.uk) in the UK as an excellent example for this approach. In this scheme, risks of anticoagulant rodenticides for predatory birds were identified and established in the early 1980s[6], and also the potential impacts of brominated flame retardants. An essential step would be to characterise potential effects under environmentally-relevant, chronic, exposure conditions. These effects can be subtle, and therefore overlooked until now, but nevertheless may significantly affect animals. Current studies show such adverse effects on behaviour and the immune system, also in our lab, however more insights are needed on the specific mechanisms of toxicity, adverse long-term impacts on organisms and population and also on species-specific sensitivity. This needs to be based on mechanistic research in order to disentwine the impacts of the chemicals from environmental and ecological variability.


Wildlife species are often charismatic with high conservation status. Chemical impacts on wildlife have evolved from 'dropping dead' to more subtle effects, which is positive. However, since these subtle effects can still adversely affect the fitness of the animals, ERA frameworks should take a step further and develop sound posteriori exposure and effect assessment to allow tailor-made considerations of chemical impacts on wildlife species, even when effects may at first sight not be evident. Further refinement of current prospective ERA procedures will not be able to cover this, so it is time to make ERA more inclusive and focus on chemicals risks for wildlife species currently not addressed. ■

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# ECETOC develops CF4Polymers

## a conceptual framework for the safety assessment of polymers

**The Centre for chemical safety assessment (ECETOC) has developed a new Conceptual Framework for Polymer Risk Assessment, known as CF4Polymers.**

Currently, the notification of polymers is not required within the EU, but an initiative between the European Commission and ECHA is carefully studying how to implement a notification process and testing requirements for PRRs (Polymers Requiring Registration). Polymers are, by nature, highly complex mixtures and are typically challenging to test and assess. Regional differences in regulatory definitions add further to the complexity, hence the need for uniform principles and guidance.

In recent years, ECETOC has played a key role in providing scientific solutions for chemical safety assessment during the introduction of European regulations on the production and use of chemical substances.

To support the current regulatory discussions around polymers, the Technical Report No. 133 has been developed by ECETOC's Polymers Task Force as a three-part series and aims to provide a scientific perspective for the safety assessment of these chemicals.
Olivier de Matos, ECETOC Secretary General, said: "This series of three reports represents a major step forward in the safety assessment of polymers. It provides consistency and is also the first time that the polymer safety assessment process not only addresses the polymer itself, but also any potential added substances."

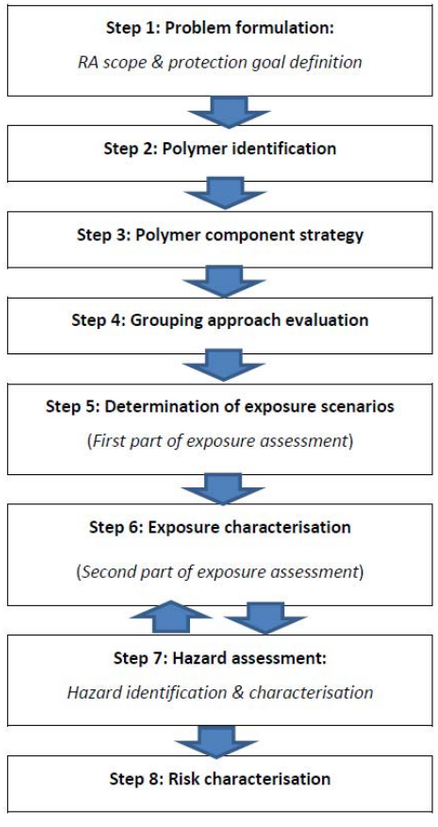
**CF4Polymers provides guiding principles for safety assessment**

The first part of the series (ECETOC Technical Report (TR) No. 133-1) presents the Conceptual Framework for Polymer Risk Assessment, also known as CF4Polymers, and provides basic guiding principles for assessing potential ecological and human health hazards and risks posed by polymer products, thus facilitating a consistent approach. The CF4Polymers lays out a state-of-the-art framework for the safety assessment of polymers, including polymer grouping and safety assessment approaches. The framework is, however, not prescriptive; it is designed to be flexible.

The work of the ECETOC Polymers Task Force was motivated by the versatility and complexity of polymers. Polymers are generally not present as mono-constituent substances, but as complex polymer products consisting of the polymeric substance (polymeric macromolecules), intentionally added substances (IAS), such as stabilisers, and non-intentionally added substances (NIAS), such as impurities. Further, there are often many actors in the life-cycle of polymer products (and articles containing polymer products) and polymer products can also change their form (chemistry and/or morphology) during the different life cycle stages.

For these reasons, conventional safety assessment approaches for chemicals may need to be modified for polymers.

CF4Polymers sets out the following eight steps for polymer safety assessment: ➜



The sequence of these steps can be adapted as necessary depending on the risk assessment needs and/or data availability. For example, it may be preferable to perform an initial hazard assessment (Step 7) before exposure characterisation (Step 6) and to finalise hazard assessment thereafter, or to determine exposure scenarios (Step 5) already during problem formulation (Step 1).
For each of the eight steps of the CF4Polymers, a detailed outline is provided for how it can be completed, accompanied by explanatory notes and illustrative examples and a comprehensive glossary providing definitions for all relevant terms. In addition, for each step, prevailing knowledge gaps are addressed.
Finally, five recommendations are made for further research. They are:

1. Identify sets of structural and/or morphological descriptors as well as physico-chemical and fate properties that are key parameters for different types of polymer products;
2. Consider prevailing technical limitations of available tools, test methods and models for polymer safety assessment;
3. Maintain the CF4Polymers as a 'living', flexible framework;
4. Establish a knowledge base to substantiate the 'polymers of low concern' (PLC) concept and to identify under which conditions the presence of specific structural alerts or physico-chemical properties poses environmental or human health hazard concerns; and
5. Develop environmentally relevant models, methods and/or criteria to assess (bio)degradation to enhance assessments of the safety assessment implications of this property.

**Review of the applicability of standard tools, test methods and models**

The second report in the series (ECETOC TR No. 133-2), advances the topic of polymer safety assessment by providing a detailed review of the applicability of standard analytical tools, *in vitro* and *in vivo* test methods and *in silico* models to assess the physico-chemical, fate, exposure-related, ecotoxicological, and toxicological properties of polymers.

The test methods and parameters that are further assessed for their relevance in hazard and/or risk assessment, in line with the recommendations of the CF4Polymers, include:

- The importance of polymer identification accuracy prior to performing tests;
- Analytical tools to assess physico-chemical properties with a focus on weight-average and number-average molecular weight, acid dissociation constant, n-octanol/water and organic carbon/water partition coefficients, solubility in water or fat, surface tension; further discussing approaches for verification of in-life exposure, i.e. analytical measurements of polymers in environmental matrices;
- Test methods addressing the environmental fate properties of biodegradation, abiotic degradation and bioaccumulation/bioconcentration;
- Environmental exposure modelling addressing the compartments of soil, wastewater treatment plants, freshwater and freshwater sediment, and marine surface water and sediment;
- Human health exposure modelling addressing both occupational (industrial and professional) and consumer exposure;
- Ecotoxicological assessments using aquatic organisms, sediment-dwelling organisms, and terrestrial organisms (soil organisms, and plants);
- Human health hazard assessments.

The authors stress that this second report is not intended as a check list for a 'tick-box approach' to hazard and risk assessment of polymers. Instead, the report includes guidance on how to identify if a specific test method might be relevant for specific types of polymers, as well as how relevant test methods might be structured in a tiered approach.

Three conceptual frameworks for assessment of polymer biodegradation, bioaccumulation and ecotoxicity are presented. These complement the first report by 'zooming into' specific steps of the CF4Polymers to show how potential for (bio)degradation, bioaccumulation and ecotoxicity might be addressed within the CF4Polymers. As with the first report, this second report also includes an extensive glossary of all key terms.

**Case studies under preparation to put CF4Polymers into practice**

Currently, a set of case studies is being prepared which will be presented as ECETOC TR. No. 133-3 to complete the trilogy. These case studies will address different components of polymer safety assessment to put the CF4Polymers into practice and to demonstrate the considerations on applicability of available tools, test methods and models that can be used to ensure the safe use of polymers.

The third part of the trilogy is expected to be made available by mid-2021. ■

**What is ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals)?**
Established in 1978 and based in Brussels, Belgium, ECETOC is a collaborative space for leading scientists from academia, governments and industry to develop and promote trusted and practical solutions to scientific challenges which ensure a safe, sustainable and healthy world.

ecetoc
WE ARE THE CENTRE FOR CHEMICAL SAFETY ASSESSMENT

# Neurotoxicity in Environmental Toxicology and Water Quality Management

*By Jasper Woutersen*

Neurotoxicity, a well-established human toxicity endpoint, is gaining traction as an endpoint of environmental toxicity. Currently, the main focus of neurotoxicological research is to develop models to assess potential effects of chemicals in humans and it has been estimated that 30% of the commercially available substances have neurotoxic potential to some extent[1]. Environmental samples typically contain a multitude of low-level compounds, being either natural, synthetic or transformation products. Besides chemicals with unknown neurotoxic potential, many of the chemicals detected in freshwater monitoring have an identified neurotoxic mode of action. For example, anxiolytic oxazepam, a psycho active pharmaceutical which is frequently found in surface water, affects the behavioural traits in planktivorous fish. Therefore, is it clear that the focus should not only be on the effect of man-made chemicals on (developmental) neurotoxicity in humans but also on the potential effects of such chemicals on other living beings.

Within the EU REACH legislation, neurotoxicity only needs to be assessed for compounds with a production above 10 tons/year. Neurotoxicity for human risk assessment is then evaluated in the standard oral 28-day or 90-day toxicity studies in rodents. When these tests give an indication of neurotoxicity, such as observations of motor activity impairment or histopathological findings in the spinal cord or sciatic nerve, more detailed tests are required (OECD 424 and 426). However, in recent years more attention and effort is directed to finding alternatives for these animal tests and, moreover, these tests only focus on human or mammalian effects. At the moment there is no European regulatory framework for eco-neurotoxicity, although when substances reach the market, a lot of these substances may end up in our environment and in our water.

Chemical water quality monitoring is traditionally based on chemical analysis of prioritized chemicals in order to be able to manage and prevent risks to human health (as about 40% of the Dutch drinking water is produced from surface water). Monitoring is also performed to manage and prevent risk to all flora and fauna that have this water as their habitat. Neurotoxic and neuroactive substances that are increasingly found in the surface water can be added to such lists, but another approach is the use of effect-based monitoring using bioassays. This is the detection of responses by environmental mixtures, such as those in surface water, in biological models developed for (eco)toxicology. There are several reasons why such responses of environmental mixtures cannot be directly translated to risks, and these models are thus applied as bioanalytical tools to detect the presence of real-world exposures based on their biologically plausible effects on specific modes-of-action like neurotoxicity. In combination with innovative chemical analyses, the responsible chemicals and risk drivers can be identified for thorough risk assessment.

At first glance, water quality management and regulatory (eco)toxicology thus could seem a bit different, but they are just on different sides of the risk spectrum. In water quality management focus is on assessing substances that are already in the environment and available on the market. Whereas in regulatory (eco)toxicology focus is more on preventing substances, with for example neurotoxic potential, from getting placed on market. Within water quality monitoring you are working with water samples that can contain many different known and unknown chemicals with a range of potential toxicological effects, dealing not only with the effect of one compound but with a mixture of compounds. In regulatory toxicology, historically, the focus is on effect of single compounds, however there is increasing attention of the potential risks of mixtures of known and unknown composition. Bringing water quality monitoring and toxicological testing together, to learn from each other and increase knowledge.

KWR recently published a Joint Research Programme (BTO) report[2] about how bioassays could be used to ➜

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investigate neurotoxicity in water quality monitoring. The report summarizes the relevant literature available on neurotoxicity tests, the national research on neurotoxicity and their applicability in water quality management. I was able to ask some questions to one of the authors of this report, Milou Dingemans. I also got into contact with some other researcher for a broader perspective. I was able to ask some additional questions to Didima de Groot, retired Senior Scientist Neurotoxicology at TNO.

**Questions to Milou Dingemans:**

**What is your background and what is your role at KWR?**

After my PhD in neurotoxicology at IRAS, I stayed on for some time as a postdoc which gave me the opportunity to collaborate with other toxicologists in international projects, mostly focusing on neurotoxicology. At KWR, my work is focused on assessing chemical risks that are relevant to drinking water in order to safeguard chemical water quality. I do this within a multidisciplinary team of toxicologists, chemists and data scientists. Our projects can have many different starting points, like questions on specific chemicals, monitoring programs, materials used in production and distribution, or specialized research on toxicology and risk assessment. This makes our work very dynamic. As a guest researcher at IRAS, I also have the opportunity to be involved in academic developments, including the application of New Approach Methods (NAM) in risk assessment, integrated approaches to testing and assessment (IATA's), defined approaches for data interpretation, and performance-based evaluation of test methods. The main focus of these NAMs is to replace or reduce animal tests.

**Why is neurotoxicity assessment within water quality monitoring and also within ecotoxicology important and getting increasing attention?**

Many (types of) chemicals with potentially neurotoxic or neuroactive properties are being found in the water system. However, it is not possible to indefinitely expand the lists of prioritized chemicals of which concentrations need to be monitored. Effect-based methods are used to look at exposures from another angle, by measuring the combined effects of environmental low-level mixtures on relevant modes-of-action. Up to now, many different *in vitro* methods have been developed for neurotoxicity testing, yet practically none of them are applied as bioassays for environmental monitoring. In ecotoxicology, there is increasing attention into sub-organismal responses, giving rise to studies exploring whether human endpoints in applied bioassays for water quality are also relevant and predictive for aquatic species (fish and invertebrates). As such, aiming for a more integrated manner of the evaluation of risks for human health and ecology.

**The report describes many different approaches that could be followed, like human or non-human primary cells, organ-on-a-chip, zebrafish or omics techniques. In the report, it is also described that more research is needed to find the most promising direction. But do you have a personal preference for a certain approach?**

There are many models and methods available, and the challenge is to select the right system to collect the information needed in each particular case. For routine water quality monitoring, it is generally not feasible to apply the most complex systems as a certain throughput is needed. In earlier research (demeau-fp7.eu), criteria for the performance and applicability of *in vitro* systems were derived that can be used to select suitable bioassays for water quality monitoring. We used these criteria to evaluate currently available neurotoxicity models. In the report we show the most promising models. A next step is needed now to put these systems into practice, to find and solve practical hurdles. In this way, we can implement bioanalytical tools for neurotoxicity.

More research is needed, however, to use bioassays for more thorough risk assessment of mixtures. My colleague Astrid Reus has a lot of experience with *in vitro* guideline studies for genotoxicity and we aim to translate these to testing strategies for genotoxic compounds in water. For neurotoxicity and other endpoints, we closely follow developments in New Approach Methods for risk assessment.

One of our main topics is also the interpretation of the data collected using these models. We have developed effect-based trigger values for several bioassays that indicate a potential risk for human health. It may be possible to refine these effect-based trigger values further if we can correct for kinetic processes by using (high-throughput) PBPK models, which is a specific interest of my colleague Anne Zwartsen. ➜

**Questions to Didima de Groot:**

**What is the main focus difference between assays that are used in water quality management and for regulatory ecotoxicology testing?**

Water quality management ensures that the water quality is fit for purpose. The analysis set can vary depending on the requirements of the source water. For example, drinking water must meet certain drinking water quality criteria. Likewise, bathing water must meet quality criteria for safe swimming. Assays may involve e.g. visual, smell, chemistry, microbiology and analysis of specific chemicals (e.g. pesticides) depending on the need. Regulatory ecotoxicity testing aims to ensure that exposure to a chemical does not harm the ecosystem. For example, there may be an exposure limit below which the chemical will not cause ecotoxicity. The use of a substance may need to be restricted by regulation if it causes ecotoxicity. Regulatory ecotoxicity testing focuses on the ecotoxicity of a single chemical. In regulatory ecotoxicity testing, base set testing considers three trophic levels (fish, daphnia, algae) and determines the acute and, if required, chronic / long-term effects, as well as the potential of a chemical to bioaccumulate; usually OECD testing guidelines are used.

**The demand for alternatives to animal testing is increasing, how far away from replacing animal testing neurotoxicological testing are we?**

ECHA published the so-called ANAA-report (non-animal approaches) (https://www.echa.europa.eu/documents/10162/22931011/non_animal_approcches_en.pdf/87ebb68f-2038-f597-fc33-f4003e9e7d7d) on the basis of which it is clear that replacement of animal experiments will not happen in the near future. The most promising alternative appeared to be the *in vitro* developmental neurotoxicity test battery. And the same is also true today. There are developments at the OECD level to write a guidance document on developmental neurotoxicity, but it is difficult to say how long it will take (should have been ready by the end of this year, but will not be). And after that, these requirements still have to be included in the regulations. Currently, the BPR (Biocidal Product Regulation), which will be updated very soon, allows the use of a battery of *in vitro* tests if they provide the same information as OECD TG 426 (Developmental Neurotoxicity Study).

The current OECD/EFSA efforts are focused on inclusion of a battery of *in vitro* assays (not replacing *in vivo* tests) into regulatory neurotoxicity testing with an emphasis on developmental neurotoxicity evaluation. It is proposed to incorporate mechanistic knowledge and data derived from *in vitro* studies to support various regulatory applications including: (a) the identification of potential DNT triggers, (b) initial chemical screening and prioritization, (c) hazard identification and characterization, (d) chemical biological grouping, and (e) assessment of exposure to chemical mixtures. Currently neuronal/glial models derived from human induced pluripotent stem cells are available, avoiding species extrapolation.  A battery of human *in vitro* tests can generate valuable mechanistic data, speeding up the evaluation of thousands of compounds present in industrial, agricultural and consumer products that lack safety data on developmental/adult neurotoxicity potential.

Recently, using semi-quantitative criteria, readiness of 17 *in vitro* DNT assays has been evaluated and most of these tests turned out to be ready for different regulatory applications.

**Is finding alternatives easier in eco-neurotoxicology than in human neurotoxicological research?**

I don't think that it is easier for eco-neurotoxicity taking into account species differences. Which species should be covered for ecotoxicity and which don't? Furthermore, ecotoxicity testing is more focused on survival and reproduction of the population than specific toxicity types. In many tests in the *in vitro* testing battery human-based cell lines are used.

Notice that despite an increasing number of reports of species exhibiting altered behaviour, neurotoxicity assessment for species in the environment is not required and is therefore not usually performed. Given the increasing number of environmental contaminants with potential neurotoxic potential, eco-neurotoxicity should also be taken into account in the risk assessment. This requires new test systems that can deal with species differences within ecosystems.

In the field, online-biomonitoring systems using behavioural information could be used to detect neurotoxic effects and effect-directed analyses could ➜

be applied to identify the neurotoxicants causing the effect. Additionally, toxic pressure calculations in combination with mixture modelling could use environmental chemical monitoring data to predict adverse effects and prioritize pollutants for laboratory testing. Cheminformatics based on computational toxicological data from *in vitro* and *in vivo* studies could help to identify potential neurotoxicants. An array of *in vitro* assays covering different modes of action could be applied to screen compounds for neurotoxicity. The selection of *in vitro* assays could be guided by AOPs (Adverse Outcome Pathways) relevant for eco-neurotoxicity. In order to be able to perform risk assessment for eco-neurotoxicity, methods need to focus on the most sensitive species in an ecosystem. A test battery using species from different trophic levels might be the best approach. To implement eco-neurotoxicity assessment into European risk assessment, cheminformatics and *in vitro* screening tests could be used as first approach to identify eco-neurotoxic pollutants. In a second step, a small species test battery could be applied to assess the risks for ecosystems.

**In the last couple of years endocrine disruption has taken high interest within the regulatory framework. From 2018 onwards a framework was introduced for testing of endocrine disruption potential of plant protection products, resulting in the need to test these prodcuts for potential endocrine disruption in both humans and non-human animals. Do you think neurotoxicity could be the next thing that needs to be tested for in the environmental risk assessment?**

Indeed, endocrine disrupting activity and properties have become current lately. Neurological effects also play a role in ED (endocrine disruption) testing: e.g. thyroid disruptors also give effects at the level of the human brain. Endocrine disruption can be seen as mode(s) of action which may cause many so-called apical effects; (developmental) neurotoxicity, effects in sexual function and fertility, developmental toxicity (malformations), cancer etc. It must be separated if the substance has ED activity OR if the substance is an endocrine disruptor, meaning it has the ED activity but also causes serious effects such as reproductive toxicity which is classified (e.g. under REACH: Substance of Very High Concern (SVHC), and can be regulated accordingly). BPR (Biocidal Products Regulation) will be published shortly with updated requirements of ED testing, as well as mandatory information on developmental neurotoxicity (OECD TG 426). Animal studies suggest that maternal exposure to EDs produced changes in rearing behaviour, locomotion, anxiety, and learning/memory in offspring, as well as neuronal abnormalities. Some investigations suggested that EDs exert effects on central monoaminergic neurons, especially dopaminergic neurons. Perinatal exposure to EDs might affect neuronal plasticity in the hippocampus, leading to impaired cognitive and memory functions. EDs also attenuate gender differences in brain development. For example, the locus ceruleus is larger in female rats than in males, but treatments with bisphenol-A (BPA) enlarge this region in males. Some EDs induce hypothyroidism, which leads to an abnormal brain development. EDs also affect mature neurons, resulting in neurodegenerative disorders such as Parkinson's disease.

Indeed, neurotoxicity (especially developmental neurotoxicity) assays could serve as sensitive indicators/biomarkers of environmental risk assessment. ■

Photo by Robina Weermeijer on Unsplash

# In memoriam: Henk Tennekes

## (Zutphen, 21 November 1950 – Winterswijk, 7 July 2020)

*Dr. Ir. HA (Henk) Tennekes*

**On the 7th of July of this year, the toxicology community lost Henk Tennekes, one of its most authentic members, who was especially well-known for his influential work on neonicotinoids. Henk suffered from pulmonary hypertension (in addition to COPD, diabetes and Parkinson's disease), a debilitating and progressive disease that severely impacts quality of life. Henk chose euthanasia instead of increasing suffering for maybe several years to come; he passed away on the 7th of July 2020.**

He was born in Zutphen on the 21st of November 1950 and obtained his MSc in Human Nutrition at the University of Wageningen in 1974. The next years, he performed his PhD research at the Shell Toxicology Laboratory in Sittingbourne, UK and was awarded his PhD degree in 1979 from Wageningen University. The title of his PhD thesis was "The Relationship between Microsomal Enzyme Induction and Liver Tumour Formation". During his PhD, he investigated the effects of the insecticide dieldrin on tumour formation in mice, including the modulating effects of environmental factors such as diet and bedding [1,2]. As a young researcher he was already very interested in the quantitative aspects of toxicology [3,4,5,6]; this interest persisted throughout his career [7,8].

After obtaining his PhD, he worked at Philipps University of Marburg (Germany), the Krebsforschungszentrum in Heidelberg (Germany), at Sandoz in Muttenz (Switzerland; currently part of Novartis) and the Research and Consulting Company (RCC) in Itingen (Switzerland). He started working as an independent consultant in 1992.

As mentioned in the first paragraph, Henk was particularly triggered by the topic neonicotinoids in his later career. These insecticides, also known as neonics or NNIs, were developed by Shell and Bayer in the 80s and 90s. They were initially hailed as miracle insecticides with limited toxicity to mammals (including humans), birds and other higher organisms. After coating the seeds intended for sowing, the substances are distributed throughout the structures of the mature plant, including the pollen. Indeed, their use effectively minimized crop destruction by harmful insects. Their toxic mechanism of action is by targeting the central nervous system of insects which leads to their paralysis and death 9. The first neonic was approved for use in the EU in 2005, while in 2013, a total of five neonicotinoids were approved as active substances to be used in plant protection products in the EU, i.e. clothianidin, imidacloprid, thiamethoxam, acetamiprid and thiacloprid.

In 2010, Henk published the book "A disaster in the making", in which he extensively discussed the risks of neonicotinoid pesticides for insects, especially bees. This book can be seen as a spiritual follow-up to Rachel Carson's "Silent Spring", a 1962 book on the environmental risks of pesticides that led to the banning of DDT for agricultural use. He was fascinated by the Druckrey-Küpfmüller equation (d x tn = constant; with ➜

d = daily dose and t = exposure time to effect). Henk argued that this equation was applicable for neonicotinoid toxicity in bees, because binding to their receptors was irreversible as well as the toxic effect. Adherence to this equation would indicate that long-term exposure to relatively low levels of insecticide can still have marked toxic effects in bees [8,10].

In 2015 the results from an integral review on the effects of neonicotinoids (the Worldwide Integrated Assessment of the Impact of Systemic Pesticides on Biodiversity and Ecosystems) were published; an international panel concluded on the basis of available evidence, including that put forward by Henk and colleagues, that it was likely that existing levels of neonicotinoid pollution had large scale detrimental effects on invertebrates including honeybees [11]. EFSA concluded in 2018 that three of these neonicotinoids should be banned altogether for outdoor use [9].

During the freelance phase of his career, Henk experienced great professional difficulties after making his opinion on neonicotinoids public; it was not a popular message which had large economic consequences for the industry and farmers. At least in the short term; in the long term the economic consequences of bee extinction would be far greater. He will be remembered not just for his important work on neonicotinoid toxicity in bees, but also for his determination to do the right thing even when this has negative personal consequences.

*In Loving Memory*

May he rest in peace and may we remember his main message: **take care of our planet.** ■

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# Why should we care about “Quantification and modelling of Fluxes of persistent organic pollutants (POPs) to Antarctic marine benthic organisms?” Here is why.

*By Artem Krasnobaev*

**After this clickbaity title I would like to do even a more clickbaity thing and send you to https://weblectures.wur.nl/ to enjoy the actual online defence of my thesis. But here is a synopsis of what it is all about.**

POPs are pollutants – both historical and currently produced – that can travel from the places of their release (e.g. in America or Asia) through the atmosphere to the most remote areas of our planet, including Antarctica. This is especially important for the Antarctic marine benthic ecosystems already endangered by climate change, as they act as the ultimate sink of these pollutants. Characterization of concentrations of POPs and forecasting their trends were the core of my research. Tough for me, but good for the environment: these concentrations turned out to be orders of magnitude lower than it had been expected from the previous studies. The toughness lied in the absence of analytical techniques with which POPs can be reliably measured at such concentrations. But in the end with the great help of our colleagues from Wageningen Food Safety Research institute (WFSR) we developed an (almost) universal method to measure POPs in numbers in which normally only dioxins are measured. The good for the environment continued with the outcomes of our toxicological study, where we tried to see changes of enzyme activity and omics in Antarctic limpets upon exposure to PCBs. The results were inconclusive, what may mean that the benthic organisms of the Antarctic seas are not as susceptive to POPs as previously thought.

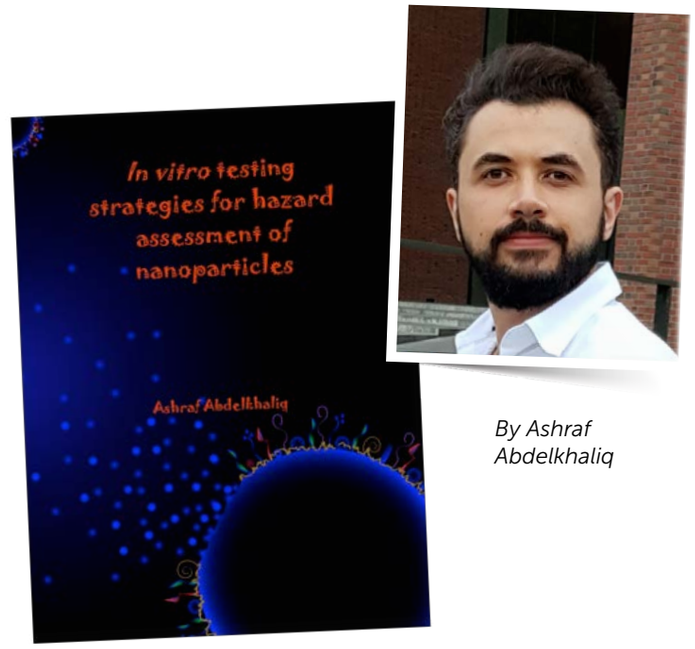
Yet sadly there are also points of concern. It is highly likely that these currently low concentrations of pollutants will increase in the future despite international efforts to decrease their emissions. In addition, it is not known how the behaviour of these pollutants and the resilience of ecosystems against them will evolve following the climate change.

Overall, it is wonderful that my results have brought more hope than despair, although the future remains yet uncertain. More research is needed ™. Scientific collaboration is required for it to catch on – and a good one of that I consider to be a recipe of success for my PhD. The inputs of my colleagues from WUR Tox, WFSR, British Antarctic Survey, Environment Canada, Australian Antarctic Program, and other places were integral to my work, and a thank you to all of them.


On the COVID note, I for one liked having my thesis defence online, as it was very efficient. No magic of a moment is needed where there is honest work. Yet, I understand that it must have been difficult to organise this event and my appreciation goes towards those who did it, especially my supervisors and my paranymphs. I really relished receiving congratulations from my former colleagues after the ceremony. ■

# *In vitro* testing strategies for hazard assessment of nanoparticles

## PhD graduation at Wageningen University




*By Ashraf Abdelkhaliq*

**On the 9th of June 2020, I have been awarded a PhD degree in Toxicology from Wageningen University. This was the result of the successful completion of the research project entitled '*In vitro* testing strategies for hazard assessment of nanoparticles' which was a collaboration between the Division of Toxicology - Wageningen University (Prof. Ivonne Rietjens and Dr. Hans Bouwmeester) and Wageningen Food Safety Research institute (WFSR, previously known as RIKILT) (Dr. Meike van der Zande). Owing to the ongoing Covid-19 pandemic, the defence was done remotely via Skype.**

### The motivation

The increasing use of nanoparticles (NPs) in a wide range of applications results in an increased likelihood of human exposure to NPs. Humans can be exposed to NPs via different exposure routes including dermal, inhalation or oral routes where the latter represents the exposure route that was considered in this project. At the present state-of-the-art, the safety assessment of NPs relies heavily on *in vivo* studies. Considering the great diversity in the types of NPs in terms of their intrinsic properties and how these can be modified by external factors, the number of laboratory animals required to evaluate the potential toxicity of all these types of NPs would be unacceptably large.

### The aim

In this project we aimed to investigate the potential of different *in vitro* methods combined with advanced analytical techniques as a screening strategy to study the toxicokinetic and toxicodynamic properties of silver (Ag) and polystyrene (PS) NPs.

### The research

The influence of the size and surface chemistry of pristine PSNPs on the protein corona formation and subsequent uptake/association and transport of these NPs through a Caco-2 intestinal cell model were studied. Furthermore, the impact of the biochemical conditions within the human digestive tract on the intestinal transport of AgNPs of different surface chemistries across an intestinal *in vitro* model of differentiated Caco-2/HT29-MTX cells was studied. An *in vitro* digestion model was used to simulate the human digestion process. AgNPs of different surface chemistries and silver nitrate ($AgNO_3$) as a source of ionic silver were used. Using ICP-MS and spICP-MS, the size distribution, dissolution, particle concentration (mass- and number-based) and total silver content of the AgNPs were characterized before and after digestion and in the apical, basolateral and cellular compartments of the Caco-2/HT29-MTX intestinal transport model.

After that, a combination of the BeWo b30 placental transport model and the embryonic stem cell test (EST) was used to investigate the capability of pristine AgNPs of different surface chemistries and aged AgNPs ($Ag_2S$ NPs) to cross the placental barrier and their capability for inducing *in vitro* developmental toxicity. Again, $AgNO_3$ as a source of ionic silver, was tested for comparison. ICP-MS and spICP-MS were used to characterize the size distribution, dissolution, particle concentration (mass- and number-based) and total silver content of the AgNPs in the apical, basolateral and cellular compartments of the BeWo b30 placental transport model at different time points. ➜

Finally, the In-Cell Western- γ-$H_2$AX assay was evaluated as an alternative *in vitro* assay to detect the potential of aged AgNPs and pristine AgNPs to induce phosphorylation of $H_2$AX, reflecting induction of DNA-DSBs, in HepG2 liver cells. $AgNO_3$ was used to quantify the effects of ionic silver. Additionally, the potential of these AgNPs to induce ROS production as a potential underlying mechanism of inducing DNA-DSBs, was assessed, both under cell-free conditions and in HepG2 cells.

**Online defence**

Having the defence and the associated graduation ceremony is something every PhD candidate is waiting for as an announcement for his/her successful completion of his/her PhD journey. In addition, the presence of your family and friends in this moment and sharing it with them is something indescribable. When the time came for my defence, the Covid-19 pandemic was already swiping the world which put a lot of restriction on having the expected ceremony and celebration. I had to make a difficult call, either to postpone the defence or to proceed and pursue my dream of defending my thesis in an online setup. I decided to move on and make this challenge a successful opportunity. But this was not going to be a success without the indescribable support from my family and my brave and amazing paranymphs. Although online defences do not have the glory of the traditional ceremonies, I must say that it was an amazing and different experience. The stress level is significantly lower as you do not have tens or hundreds of eyes staring at you, very quiet environment and having the support of your paranymphs (with consideration of social distancing rules). Additionally, I was blessed to have the opportunity to celebrate online with colleagues and friends right after being awarded the degree where I received their congratulations, nice gifts and two bouquets of flowers from both WFSR and Toxicology department. It was a special and unforgettable experience. ■

# AIO toxafette - Lora-Sophie Gerber

*By Lora-Sophie Gerber, Institut for Risk Assessment Sciences (IRAS), Faculty of Veterinary Medicine, Neurotoxicology research group*

## 1. Can you introduce yourself?

My name is Lora-Sophie Gerber and I come from the Black Forest in southern Germany. For my bachelor's I studied Nutrition Science in Jena and after traveling for a year I continued doing my master's in Toxicology in Düsseldorf. During my master's internship, I was introduced in the research field of Alzheimer's disease and became fascinated with the brain - a highly vulnerable and at the same time well-protected organ, which essentially determines who we are. Not planned and slightly lucky, I ended up in lovely Utrecht starting my PhD at the Institute for Risk Assessment Sciences in October last year. The aim of my PhD project, supervised by Prof. Flemming Cassee and Dr. Remco Westerink, is to assess the neurotoxic potential of traffic-derived ultrafine particles (UFPs).

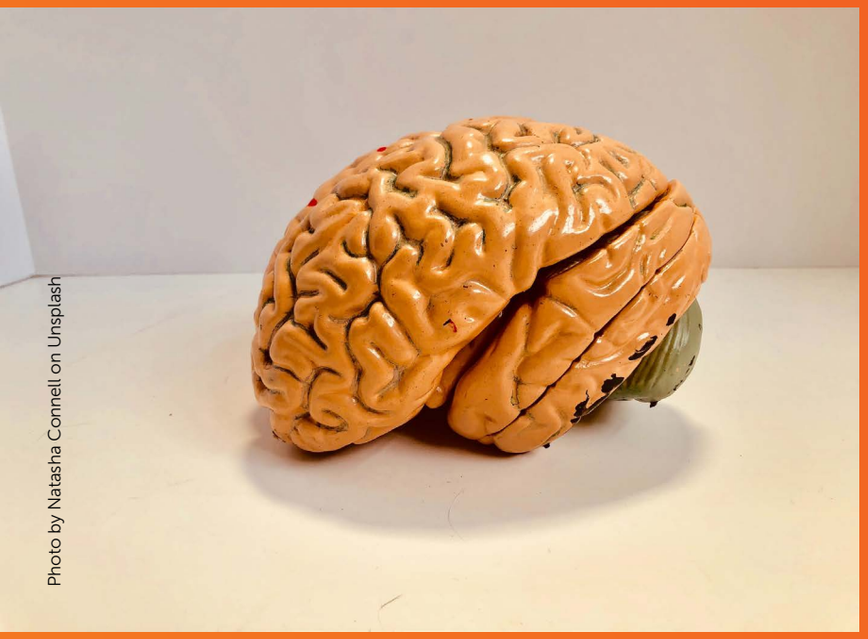


Photo by Natasha Connell on Unsplash

## 2. How would you explain the subject of your research to a layperson?

Due to their extremely small size (< 100 nm in diameter), UFPs can translocate through epithelial barriers and reach secondary target organs such as the brain. There is evidence that traffic-derived ultrafine particles (UFPs) are involved in neurodegenerative processes and contribute to the development and progression of neurodegenerative diseases. Only little is known about which types of particles are the most harmful one and what kind of effect they have on brain development and health. Therefore, one aim of my PhD project is to identify neurotoxic traffic-derived UFPs and to characterize their neurotoxic effects. Another focus of my research is to assess the indirect effect of traffic-related UFPs on the brain. The lung displays the primary target organ of ambient UFPs. The cellular response of the pulmonary cells, e.g. release of proinflammatory signals, can also affect brain function. To study this physiological relevant exposure scenario, we collaborate with researchers at RIVM and combine their lung exposure model (air-liquid interface system, ALI) with our neurotoxicity screening method (microelectrode array, MEA).

## 3. What was your motivation to start a PhD program?

By nature, I am a very curious person and I love to try new things. When I actively started doing research as a master student, I discovered how much I like planning and performing experiments. I realized that I enjoy pursuing a question and considering all eventualities. That's why I thought that doing a PhD is the best way to pursue this passion. Even if it sometimes is fairly challenging, waiting for the result can be as exciting and full of anticipation as preparing for a long journey.

## 4. Why did you choose a subject in toxicology?

I chose a subject in toxicology because I like the fact that it is a very interdisciplinary field of research. In addition to the questions of what effects a compound has on certain organ systems and the underlying mode of action, one must also consider the exposure scenario, the fate of the compounds in the body and the impacts of primarily induced effects on the whole organism. I think in order to get the big picture, toxicologists ultimately have to think a little bit more outside the box than other researchers. →

**5. What is the best advice that you have received as a PhD student or would like to give to another PhD student?**

1) Never be afraid to ask for help
2) Plan, but stay flexible

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

Over the past year I have met many inspiring and helpful people at work. Instead of leaving on time, I always enjoy having a coffee and nice conversations with my colleagues, even if it means I have to stay at work a little longer. I think this helps me a lot to combine my work and my private life. As a balance to my PhD project I like to do gardening and outdoor activities like camping or boat trips in my free time. Also, this summer I spent many evenings outside with friends, barbecuing at the campfire and having a few drinks. Even though I sometimes stay at work for a very long time, these are activities that I have always been able to integrate into my daily life. So no, I don't have to make any decisions, or at least I don't feel like I have to.

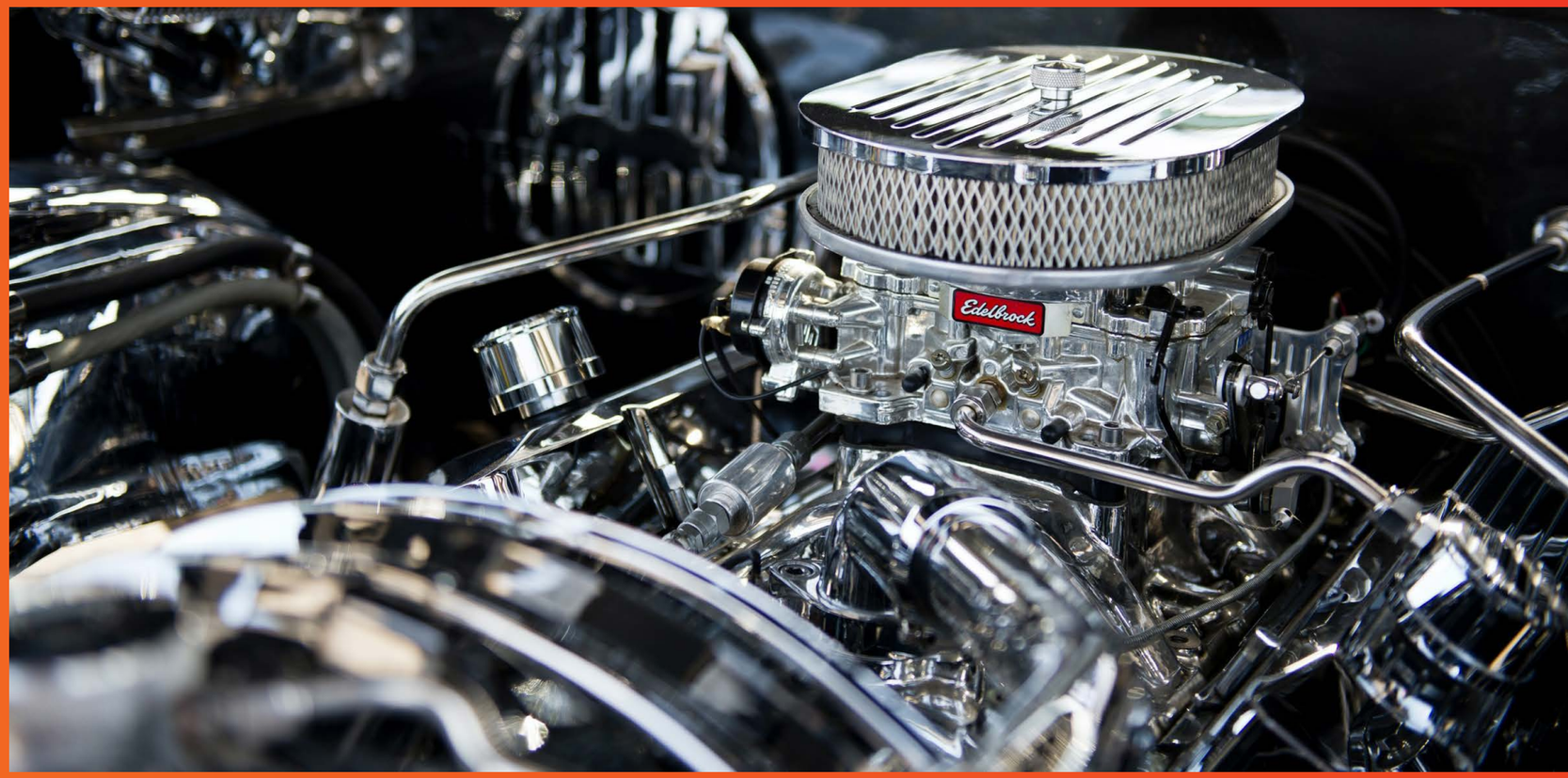
**7. What is the biggest challenge for you in doing PhD research?**

The biggest challenge for me is not to lose focus while reading literature and writing my paper. Besides, the research area of traffic-related air pollution and ultrafine particles is new to me, and I easily get lost in all the new information. Not to mention the chemical and analytical stuff. Help!

Also, never before have I dealt with engine technologies or the different components and compositions of various types of fuel and oil, etc. Before starting this project, I only knew that there are engines that run on gasoline or diesel and that you better not mix them up.

**8. Please answer the question from the last toxafette PhD-candidate: Knowing that you did your studies in Germany: are there differences in the way Dutch and German academia interact with each other? And if so, what difference was most striking for you?**

I am not under the impression that there are many differences in the way Dutch and German academia interact with each other. In general, the Netherlands and Germany don't differ too much and people are pretty similar. Also, I think the interaction in academia differs between institutions and it depends more on the people working there than on cultural conventions. The way researchers actively create their working environment and how they deal with their colleagues on a daily basis influence the interaction more than Krokets and Weißbier.

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# New Fixed Format for the NVT Travel Grant Reports

**As presented at the virtual NVT general meeting in June, we have developed a new format for the NVT travel grant reports which is aimed at guiding the submissions and providing TCDD readers even more interesting reports.**

Naturally the reports need to have the full name of conference, location (city, country) and date of the conference as well as the name and affiliation of the author. In brief, in a maximum of 600 words, the reports should:

1. Describe the author's own presentation at the conference
2. Describe the three most interesting learning/insights
   – Summarize the most interesting other presentation(s)
   – Researchers met at the conference that proved to be a source of inspiration or could provide useful inspiration/collaboration for future research
   – Describe a valuable new technique that was presented
3. Give a scientific "take home message" from this conference
4. Finally, describe in what ways the conference organization, and the author, strived to make the conference a climate neutral event

Photo by Ben Kolde on Unsplash

REGISTRATIE CIE

## Inschrijving register

| Voorletters | Achternaam | Datum inschrijving | Datum afloop registratie |
|---|---|---|---|
| A.P. | Walczak | 20-05-20 | 20-05-25 |
| A. | Al-malahmeh | 20-05-20 | 20-05-25 |
| A.M.M. | Kraaijvanger | 20-05-20 | 20-05-25 |
| S. | Suparmi | 20-05-20 | 20-05-25 |
| M. | Marinkovic | 10-06-20 | 10-06-25 |

## Inschrijving TiO

| Voorletters | Achternaam | Opleider | Datum inschrijving |
|---|---|---|---|
| K. | Niermans | Prof.dr.ir. I.M.C.M. Rietjens | 20-05-20 |
| J.J. | Nugteren-van Lonkhuyzen | Prof.dr. M. van den Berg | 20-05-20 |
| P.N.H. | Wassenaar | Prof.dr. M. van den Berg | 20-05-20 |
| L.C. | Jager | Prof.dr. D.J. Touw | 20-05-20 |

TERUG NAAR INHOUD

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