TCDD

TOXICOLOGIE

NUMMER 3
OKTOBER 2021

SPECIAL THEME

EU CHEMICALS STRATEGY

- **TOWARDS A TOXIC-FREE ENVIRONMENT:** the ambition of the European Commission
- **CHEMICALS STRATEGY FOR SUSTAINABILITY:** what are the practical effects?
- **EU CHEMICALS STRATEGY IN THE HOT SEAT**
- **A REVIEW OF THE HERZLER ET AL. CRITICAL PAPER ON THE EU CHEMICALS STRATEGY FOR SUSTAINABILITY**
- **CASE STUDY:** safe by Design to avoid a regrettable substitution

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Colofon

Toxicologische Communicatie, Data en Documentatie

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Editorial

Dear reader,

As the leaves slowly begin to fall and we enjoy some last days of sunny weather, most of us have returned from well-earned holidays and returned to work (hopefully) rested and ready for some new (toxicological) challenges. For me personally, it was a special summer, as my partner and I got married. Also, I found myself ready for new challenges, as I recently took on a new role as member of one of the subcommittees of the Health Council. To preserve some precious free-time, I will therefore say goodbye to the editorial board of the TCDD and this will be the last issue I will have worked on. It's been quite a ride these last 10 (or is it 11?) years as an editorial board member and I have enjoyed working on each edition (although I must admit I missed out on some too over the years). Thanks to all my wonderful fellow-TCDD members (present and past) who kept me enthusiastic all this time! On the bright side: this opens up a spot within the editorial board, so if you are interested to take part in this inspiring team, please contact them at redactie@toxicologie.nl.

Having said that, I think again we managed to prepare a really interesting issue for you to read. Our Theme is the European Chemicals Strategy for Sustainability and throughout this issue we provide you with some background information on this strategy that was published by the European Commission in October 2020. Moreover, we present you with different views on these EU ambitions and an interesting case study on BPA: "Safe by Design to avoid a regrettable substitution". And if after all this you feel like learning more on this hot topic, please check out the newest PET course on Current Topics in Toxicology, in which Safe by Design is one of the three main topics. Next to these special theme pieces, we will fill you in on all the toxicology in the media you might have missed while you were on holiday and as always, you can find a new Toxafette, written by Nienke Ruijter, PhD Student Nanotoxicology at RIVM National Institute for Public Health and the Environment as well as a Proefschrift Promopraatje by Rajinder Gupta. Please also note the announcements for upcoming meetings and events and don't forget to read-up on this year's digital NVT meeting, in case you missed it (or just want to re-visit it). And there is even more, but I will leave you to it to find that out and hope you enjoy our latest issue.

Although goodbyes are always bitter-sweet, I look forward to receiving the next TCDD in my mailbox and being surprised while finding out what's in!

Martje van de Loo

Photo by Jess Bailey on Unsplash

News from the board

Welcome to the October 2021 issue of TCDD! We hope that everyone had a good summer and a good start to the new academic year. Things are sure looking a lot different than one year ago, with the wonderful prospects of seeing colleagues and students face to face again! The first 'physical' NVT meetings are being planned, like the NVT sections Genetic Toxicology and DART symposium on "Applications of innovative stem cell technologies in genetic toxicology, teratology and reproductive toxicology" at Charles River Laboratories in Den Bosch on November 18, 2021. And of course we are really looking forward to a 'live' NVT annual meeting and member assembly next year at the Reehorst in Ede. Please save the date: **May 11-12, 2022**.

We look back on an excellent virtual annual meeting of the NVT on June 9-10, 2021. It was really one of the best virtual meetings I have attended in the last 1.5 years of working from home, thanks to the great platform offered by in3solutions and the exciting programme of speakers and poster presenters developed by our organizing committee around the topic '"The (r)evolution of toxicological models – how to address safety in target species." Many thanks to the organizing committee, consisting of PhD candidates (Gina Mennen, Christy Tulen, Lennart van Melis, Charlotte Hoogstraten, Victoria de Leeuw, Leonie Fransen) and NVT Board members (Paul Jennings, Martijn Rooseboom, Suzanne Heemskerk, Peter Theunissen). Also, our compliments to the many excellent (guest) speakers at the conference, including the winners of best platform and poster presentations. Congratulations to Dr. Anke Tukker for winning the 2020 Joep van den Bercken PhD prize for her dissertation entitled 'Exciting Models: Exploring the applicability of human neuronal cell models for *in vitro* neurotoxicity screening and seizure liability assessment". Please read further in this issue for a detailed report of the annual meeting!

The June 2021 Member Assembly meeting summarized a number of important activities in 2020, including the launching of the online re-registration system and new/renewed PET courses in 2021. For more information, please refer to the minutes of the meeting which were circulated on June 22nd, and the annual report which can be found on the TCDD site. We said goodbye to two board members in June; many thanks to Suzanne Heemskerk and Nicole Nijhuis for contributing so much of their spare time and energy to our society. We welcome Yvonne Staal as member secretary and Hans Bouwmeester as general board member and look forward to working with them!

Enjoy this issues of the TCDD dedicated to an important and timely theme: the EU Chemicals Strategy.

Kind regards, on behalf of the NVT board,

Juliette Legler

president NVT

SECTIONS GENETIC TOXICOLOGY AND DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY

Application of innovative stem cell technologies in genetic toxicology, teratology and reproductive toxicology

Date: Thursday November 18, 2021
Time: 13:30 – 17:00
Location: Charles River Laboratories, Den Bosch / Online
Cost: Free
Registration: please send an email to Josianne Theuns: j.theuns-vanvliet@erasmusmc.nl

In the last decade, stem cells have been the subject of increasing scientific interest because of their utility in numerous applications. Recent progresses in the field of Induced Pluripotent Stem Cells (iPSCs) have opened up many fantastic opportunities for research into new therapeutic possibilities but also in toxicology. iPSCs are the cells which are reprogrammed from somatic cells using different transcription factors. Stem cells, including iPSCs possess unique properties of self renewal, they can be continuously cultured in an undifferentiated state. In addition, they can be differentiated giving rise to more specialized cells of the human body such as heart, liver, bone marrow, blood vessel and nerve cells. Therefore, stem cells are an important new tool for developing unique, *in vitro* model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans.

In genetic toxicology, stem cells have been used for a long time in mutagenesis and genome stability research. Due to their stable diploid genome and high replication rate, stem cells have been applied extensively for mutation analysis and mutation fingerprinting, gene targeting, CRISPR-mediated gene modifications and genome stability research. More recently, mouse embryonic stem cells have been in various toxicogenomics studies as well as in the ToxTracker reporter assay for genotoxicity testing.

In the last years, a great deal of research has revolved around the implementation of stem cells for developmental toxicity testing. Differentiation of mouse embryonic stem cells has been used to test the developmental toxicity. The mEST was validated by the European Center for the Validation of Alternative Methods and was able to correctly categorize 78% of tested teratogens. The availability of human induced pluripotent stem cells has spurred the development of assays for developmental toxicity testing. Examples of some of the assays that have been developed using iPSCs are emerging.

This fall, the genetic toxicology and teratology and reproductive toxicology sections of the NVT will organize a joint symposium about the state-of-the-art developments and applications of stem cells in toxicology.

PROGRAM

	REGISTRATION
13:30 – 13:45	**OPENING (CHAIRS NVT GENETOX AND DART SECTIONS)**
13:45 – 14:15	**Mutational spectra and mutational signatures: insights into cancer aetiology and mechanisms of DNA damage and repair** Prof. Dr. David Phillips *Faculty of Life Sciences & Medicine* *King's College London, United Kingdom*
14:15 – 14:45	***Animal-free assessment of developmental neurotoxicity in vitro using stem cells*** *dr. Victoria de Leeuw* *RIVM, The Netherlands*
14:45 – 15:15	**Development of a novel Human Stem Cell-Based Biomarker Assay for *in vitro* assessment of Developmental Toxicity** *Dr. Amer Jamalpoor* *Toxys, The Netherlands*
15:15 – 15:45	**COFFEE BREAK**
15:45 – 16:30	**Round table discussion and workshop** "Applications of NAMs and new strategies in genetic and developmental/reproductive toxicity assessment
16:30 – 16:40	**CLOSING REMARKS**
	DRINKS

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Mutational spectra and mutational signatures: insights into cancer aetiology and mechanisms of DNA damage and repair

By *Prof. David H. Phillips. Department of Analytical, Environmental and Forensic Sciences, School of Public Health and Environmental Sciences, Faculty of Life Sciences and Medicine, King's College London*

Reporter gene assays, in which a single mutation from each experiment can contribute to the assembly of a mutation spectrum for an agent, have provided the basis for understanding the mutational processes induced by mutagenic agents and for providing clues to the origins of mutations in human tumours. More recently exome and whole genome sequencing of human tumours has revealed distinct patterns of mutation that could provide additional clues for the causative origins of cancer.

This can be tested by examining the mutational signatures induced in experimental systems by putative cancer-causing agents. Such signatures are now being generated *in vitro* in a number of different mutagen-exposed cellular systems. Results reveal that mutagens induce characteristic mutation signatures that, in some cases, match signatures found in human tumours. Proof of principle has been established with mutational signatures generated by simulated sunlight and aristolochic acid, which match those signatures found in human melanomas and urothelial cancers, respectively. In an analysis of somatic mutations in cancers for which tobacco smoking confers an elevated risk, it was found that smoking is associated with increased mutation burdens of multiple different mutational signatures, which contribute to different extents in different tissues. One of these signatures, mainly found in tissues directly exposed to tobacco smoke, is attributable to misreplication of DNA damage caused by tobacco carcinogens. Others likely reflect indirect activation of DNA editing by APOBEC cytidine deaminases and of an endogenous clock-like mutational process. The results are consistent with the proposition that smoking increases cancer risk by increasing the somatic mutation load although direct evidence for this mechanism is lacking in some cancer types. Thus next generation sequencing of exomes or whole genomes is providing new insights into processes underlying the causes of human cancer.

Animal-free assessment of developmental neurotoxicity in vitro using stem cells

By *Dr. Victoria de Leeuw. Centre for Health Protection, RIVM National Institute for Public Health and the Environment*

There is a high demand for methods replacing developmental neurotoxicity testing in animals to improve human risk assessment of compounds. Because of the complex nature of brain development, multiple *in vitro* and *in silico* tests are needed that should be combined in a testing strategy.

Within this major endeavour, this work focused on the characterisation and application of two animal-free *in vitro* tests based on embryonic stem cells for the assessment of compounds that are potentially toxic to the developing brain. mESTn mimics parts of very early neural cell differentiation of the neural tube and early hindbrain and spinal cord, and presents a wide array of different cell types along the ectodermal and neural cell lineage. hNPT mimics later developmental processes such as neuron and astrocyte differentiation, axon guidance and synaptic connectivity. These tests can, in combination with other animal-free tests, provide experimental data that can be used as part of a testing strategy to make predictions about if and how compounds may be harmful to normal neural development.

ReproTracker: A Human Stem Cell-Based Biomarker Assay for *In vitro* Assessment of Developmental Toxicity

By *Dr. Amer Jamalpoor. Toxys B.V.*

Testing for developmental toxicity according to the current OECD guidelines requires large numbers of animals, making these tests very resource intensive and time-consuming, as well as raising ethical concerns. Over the past years, several alternative *in vitro* assays have been developed, but these often suffer from low predictability and lack of mechanistic information.

Here, we present ReproTracker, a human induced pluripotent stem cell (hiPSC)-based biomarker assay that follows the differentiation during early embryonic development. The hiPSCs were directed to differentiate into three germ layer-specific cell types, hepatocytes, cardiomyocytes, and neural rosettes. The differentiation processes were followed by morphological profiling and expression patterns of the cell-specific biomarkers. In this system, a decrease in the expression of the biomarker genes and morphology disruption of the differentiated cells following compound treatment indicated teratogenicity.

The assay was validated with over 40 well-known *in vivo* teratogens and 20 non-teratogenic compounds at non-cytotoxic concentrations. In ReproTracker, *in vivo* teratogenic compounds markedly disrupted morphology and decreased the expression pattern of the biomarker genes in at least one of the three cell types. Non-teratogenic chemicals generally had no effect on the morphology of the differentiated cells, nor on the expression of the biomarker genes. Compared to the *in vivo* classification, the assay achieved high accuracy (85%), sensitivity (85%), and specificity (84%).

In conclusion, ReproTracker is a state-of-the-art *in vitro* assay that is able to identify the teratogenic potential of new chemicals and drugs with high accuracy and provide a signal as to the likely outcome of *in vivo* test systems. The assay can best serve as an early phase teratogen screening platform, or as a late phase verification for animal testing outcomes.

REPROTRACKER®

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Uniting in toxicology

It is with great pleasure that the Dutch Society of Toxicology (NVT) is inviting you to the XVIth International Congress of Toxicology in Maastricht, the Netherlands, from the 18th to the 21st of September 2022. This congress is jointly organized with International Union of Toxicology (IUTOX) and the European Society of Toxicology (EUROTOX), and unites the best of toxicological scientists worldwide in the historical city of Maastricht.

When we chose 'Uniting in Toxicology' as theme of the congress, we could hardly have imagined how appropriate it would be and how much we would be longing to re-unite after a long period of separation due to the COVID-19 pandemic. In order to make this re-union a memorable and unforgettable event, we have set-up an inspiring scientific programme and prepared a warm welcome in a lovely city and offers plenty opportunities to meet and greet in the nicest places you can imagine.

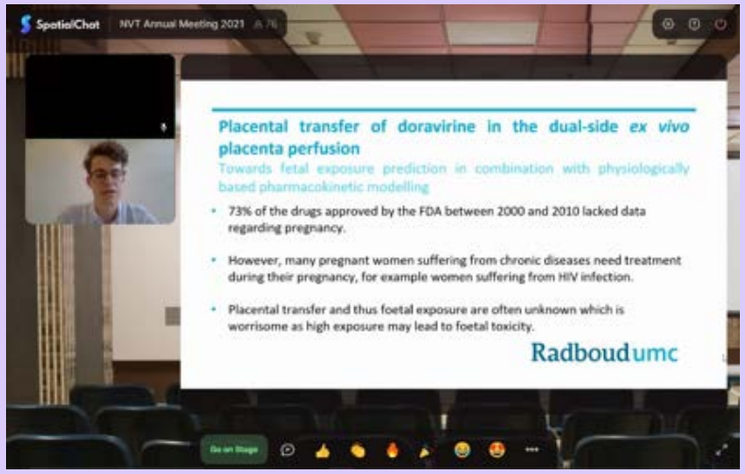
We have managed to create an exciting scientific programme in which the state of the art is presented as well as reflections on great achievements in recent years. We pay special attention to the early career toxicologist by giving them a special platform for oral communications.

The main venue of the conference is the Maastricht Exposition and Conference Centre (MECC), which has recently been completely renovated and has ample space to accommodate large groups for our keynote lectures, debates and exhibitions as well as many breakout rooms for smaller symposia and workshops. The programme will have well-balanced inputs from academia, industry and regulators and will challenge you to interact and participate in lively debates and discussions. In a smaller side-programme we aim to step out of our regular conference setting to unite in local pubs and bars with citizens and international students and discuss how toxicological research can contribute to safe products, environmental protection and a more sustainable society.

We cordially invite you to come to the ICT2022 in Maastricht and to reunite with all your colleagues in Toxicology!

Report NVT Annual Meeting 2021

This year's annual meeting of the Netherlands Society of Toxicology was held on June 9 and 10. The theme of the meeting was "The (r)evolution of toxicological models – how to address safety in target species". The two-day event was attended by almost 150 participants: professionals, PhD candidates and students from academia, industry and governmental organizations. Due to the COVID pandemic, last year's meeting had to be canceled and this year's meeting was hosted online, using the custom-made online platform SpatialChat. This platform was chosen since it enabled not only the regular (keynote) lectures, but also interaction between participants in virtual coffee rooms and during the interactive poster sessions. The conference was opened by Prof. Dr. Paul Jennings, followed by the first keynote lecture from Prof. Dr. Aldert Piersma.

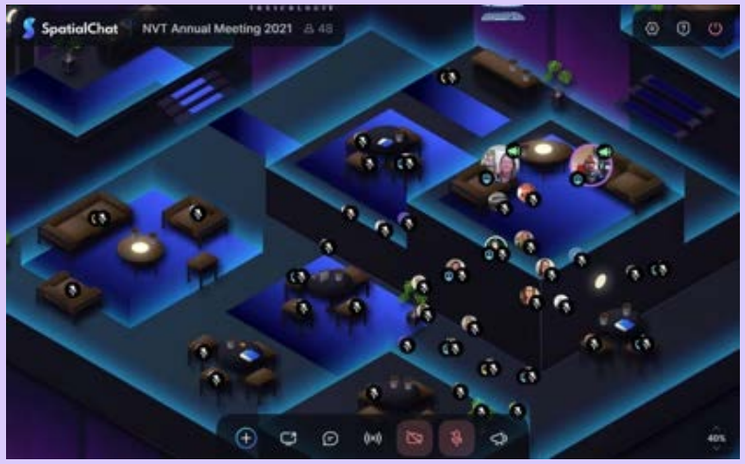


During this interactive kick-off entitled "From evolution to revolution in human chemical safety assessment", **Prof. Dr. Aldert Piersma** (IRAS/UU) showed that the Adverse Outcome Pathway framework is a useful tool to describe toxicity pathways and to select critical key events that could be monitored in dedicated *in vitro* assays. He also introduced initiatives such as the Virtual Human and the Dutch Transitional Program for Innovation without the use of animals (TPI). The interactive mentimeter part was well received by the audience who expected that no animal experimentation will be needed for chemical risk assessment within 20 years.

After a break, and possibility to network within the SpatialChat environment, the second keynote lecture was presented by **Dr. Rob Stierum** (TNO), "Reuse of existing data in human safety assessment. *In silico* and metadata analysis approaches for hazard identification and characterization". During this lecture, he showed how reanalysis of data can improve hazard evaluation and he introduced the development of *in silico* and metadata analysis approaches.

Next, the Joep van den Bercken prize 2019 was awarded to **Dr. Carin Lunenburg** who presented her thesis about: "Personalized medicine of fluoropyrimidines using DPYD pharmacogenetics." The first interactive poster session of this meeting started with the themes: *In vitro* stem cell models, *In vitro* neuronal toxicity models, Zebrafish and *in vivo* exposure models, and *In silico* and PBK modelling. In the afternoon participants who subscribed could attend one of the workshops: abstract writing, presentation skills and PROAST BMD modelling. The undergraduate speed presentations were given by Amber Mater (VU Amsterdam), Julia Meerman (Radboudumc), Rik van Dellen (Utrecht University), Jiaqi Wang (Radboud University), Lora-Sophie Gerber (IRAS/Utrecht University), Shan Wang (Maastricht University) and Damian Roelofsen (Radboudumc).

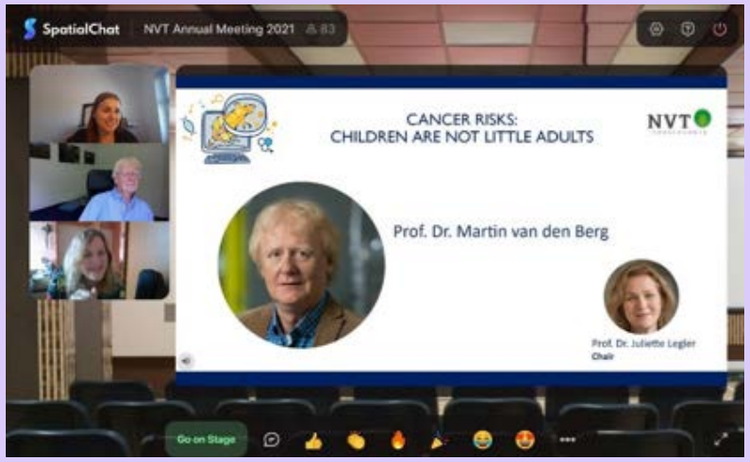
The first day was closed in the form of a College Tour where **Dr. Peter Theunissen** (CBG) interviewed **Dr. Chantal**



Smulders (Shell) on her steps in her career from the past to the future. SpatialChat was an excellent environment for the audience to ask their questions as well. The College Tour ended with a take-home message from Dr. Chantal Smulders: "Pursue your goals to your personal purpose in life and set a high ambition".

Afterwards it was time for relaxation with the cook-a-long dinner for gnocchi with pesto presented by **Victoria de Leeuw**. The Pubquiz was won by the Toxoholics team, who won a beer and bitterballen voucher. Shishani and her band closed the evening of the first successful virtual day.

The second day opened with the third keynote lecture by **Prof. Dr. Greet Schoeters** (UAntwerp) entitled: “Human biomonitoring: a step into the real world of human exposures”. The importance of making human biomonitoring data available for risk management as well as to the public in order to empower public awareness and create broader understanding towards chemical risks and prevention was highlighted in this lecture, which can also be applied in molecular epidemiology.



Thereafter, again a moment was scheduled for the interactive poster presentations focusing on the themes: 3D *in vitro* exposure models, 3D *in vitro* models, *in vitro* bioassay development, and safety assessment. Subsequently, the PhD platform presentations were scheduled in which final year PhD candidates Charlotte Hoogstraten (Radboudumc), Laura Samrani (RIVM) and Megan Houweling (Amsterdamumc) got the opportunity to present their work.

In parallel, a session was organized by **Dr. Nico van den Brink** (WUR) and **Dr. Milo de Baat** (KWR) in which they together with **Dr. Mathijs Smit** (Shell) talked about Target species: how to protect them in the environment.

After the (network) lunch it was time for the NVT business meeting or parallel the News café in which two recent news topics Microplastics and Parkinson’s disease and pesticides were (scientifically) discussed by an expert in the field, respectively **Dr. Giulio Giustarini** and **Dr. Esther de Jong**, followed by a public discussion.

Next, the Joep van den Bercken prize 2020 was awarded to Dr. Anke Tukker who presented her thesis about: EXCITING MODELS: Exploring the applicability of human neuronal cell models for *in vitro* neurotoxicity screening and seizure liability assessment.

The next session discussed The (r)evolution of human safety assessment from various points of view by **Dr. Remco Westerink** IRAS/UU), **Dr. Arne van Schanke** (Certara) and **Dr. Marjolein Wildwater** (Vivaltes).

At last, during the Grande finale Emeritus Professor in Toxicology **Prof. Dr. Martin van den Berg** shared his thoughts about the sensitivity of children to carcinogenic substances and related risk assessment issues.

The NVT annual meeting 2021 was closed by the president of the NVT **Prof. Dr. Juliette Legler** who also presented the prizes: PhD platform presentation prize to Megan Houweling (Amsterdamumc), Speed presentation prize to Lora-Sophie Gerber (IRAS, UU), PhD poster prize to Charlotte Hoogstraten (Radboudumc) and, MSc student poster prize Damian Roelofsen (Radboudumc).

Overall, even though we were not able to physically meet and greet each other, the organizing committee looks back on a great virtual meeting, which was also much appreciated according to the attendant’s evaluation, with an overall grade of 8! The committee would like to express their gratitude once more to everyone directly and indirectly involved in organizing and participating in NVT2021. All together, we made it a very big success!

Next year’s annual meeting will be organised by the NVT board members Paul Jennings, Martijn Rooseboom, and Peter Theunissen. The PhD organising committee consists of Nienke Ruijter, Tessa van Tongeren, Joyce van der Heijden and Annemijne van den Berg.

So save the date: **May 11-12, 2022, the Reehorst, Ede**! Any input or ideas can be emailed to nvtmeeting@gmail.com!

Towards a toxic-free environment:
the ambition of the European Commission

By *Hedwig Braakhuis*

Chemicals Strategy

About one year ago, the European Commission (EC) published its Chemicals Strategy for Sustainability: Towards a Toxic-Free Environment. It is a first step towards the zero-pollution ambition for a toxic-free environment announced in the European Green Deal. The Chemicals Strategy aims to ensure better protection of human health and the environment from hazardous chemicals, to boost innovation for safe and sustainable chemicals and to enable the transition to chemicals that are safe and sustainable by design.

One of the key actions in the strategy is to ban the most harmful chemicals in consumer products. Chemicals should be safe and sustainable by design (SSbD). The long-term ambition is to create a toxic-free environment. The strategy states that new chemicals and materials must be inherently safe and sustainable. The use of substances of concern should be minimized and substituted as far as possible. There is special attention for endocrine disrupting (ED) chemicals. The EC proposes to establish a legally-binding hazard identification of ED, ban them from consumer products and introduce them as substances of very high concern (SVHC).

The European Commission is developing criteria for SSbD chemicals and will develop methodologies for chemical risk assessment that take into account the whole life cycle of substances, materials and products. Also on a national level, there are ambitions to use chemicals that are sustainable and recyclable by design by 2050.

Safe-and-Sustainable-by-design

By funding Horizon Europe projects, the EC aims to accelerate the development of chemicals that are SSbD. The idea behind SSbD is that innovators and product developers take safety and sustainability into account already in the design phase of a product. The challenge is to develop products with the desired functionality without any toxicity during its life-cycle (production, use and waste). Several research projects are currently developing frameworks and tools to help industry to apply SSbD for their products. In addition, the EC funds a public partnership for the assessment of risks from chemicals (PARC). In PARC, member states will cooperate to contribute to a toxic-free environment. This includes the operationalization of the SSbD criteria that the EC will define.

To achieve the use of chemicals and products that are SSbD, one needs to know which properties of a product might induce toxicity, so these properties can be avoided. This includes understanding how properties might change throughout a products life-cycle and how this might affect toxicity. For example, asbestos fibres can cause mesothelioma due to their length, rigidity and biopersistency. From a SSbD perspective, fibres that are long, rigid and biopersistent should not be used.

As there are many knowledge gaps in understanding the relation between substance properties and toxicity, applying SSbD will include performing *in silico* modeling and performing *in vitro* assays to predict potential toxicity. Upcoming methods, such as machine learning, might also help to further unravel the relation between substance properties and toxicity. Therefore, SSbD might also help to enhance the use and acceptance of alternative methods to assess the hazard of substances.

There are some debates whether exposure is also part of SSbD. According to the Chemicals Strategy, no harmful

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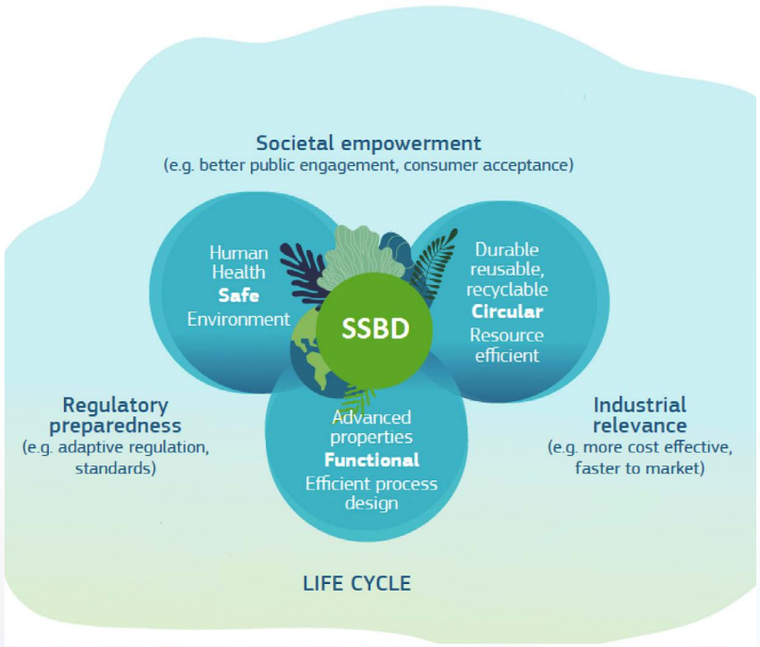
substances should be used. However, one could argue that in case there is no exposure of potentially toxic substances to humans or the environment, the substances could be used if needed for a specific functionality. Preventing exposure could also be a SSbD strategy.

Taken together, the ambitions of the EC might be difficult to meet as it might be a challenge to develop products that remain functional without any hazard to human or environment throughout the life-cycle. At the same time, aiming for a toxic-free environment will keep us all working further towards the improvement of products and the improvement of risk assessment, including the use of (innovative) alternative methods.

In this special theme on the EU Chemicals Strategy, different views on these EU ambitions are discussed.



EU Chemicals Strategy in the Hot Seat

By *Barae Jomaa*

Toxic-free and pollution-free ambition is being labelled by some as “hazard-based” and lacking in “sound scientific evidence”.

The EU Chemicals Strategy has suggested a path towards a toxic-free and pollution-free environment[1]. While it is a noble goal, a lot of chemicals are harsh by design as they need to fulfill specific functions. Exposure is just as important as the toxicity hazards that are feared so much. Despite the “toxic-free” catchword, the report stops short of defining the term toxic-free. Clearly, a truly toxic-free environment is not possible - or even desirable - considering our reliance on highly functional chemicals in order to sustain world economies and maintain our current standard of living. Without a clear definition of the toxic hazards being prioritized as substances of concern and without placing greater emphasis on exposure as part of a comprehensive risk assessment, the EU Chemicals Strategy for Sustainability has understandably received some backlash within the scientific community[2].

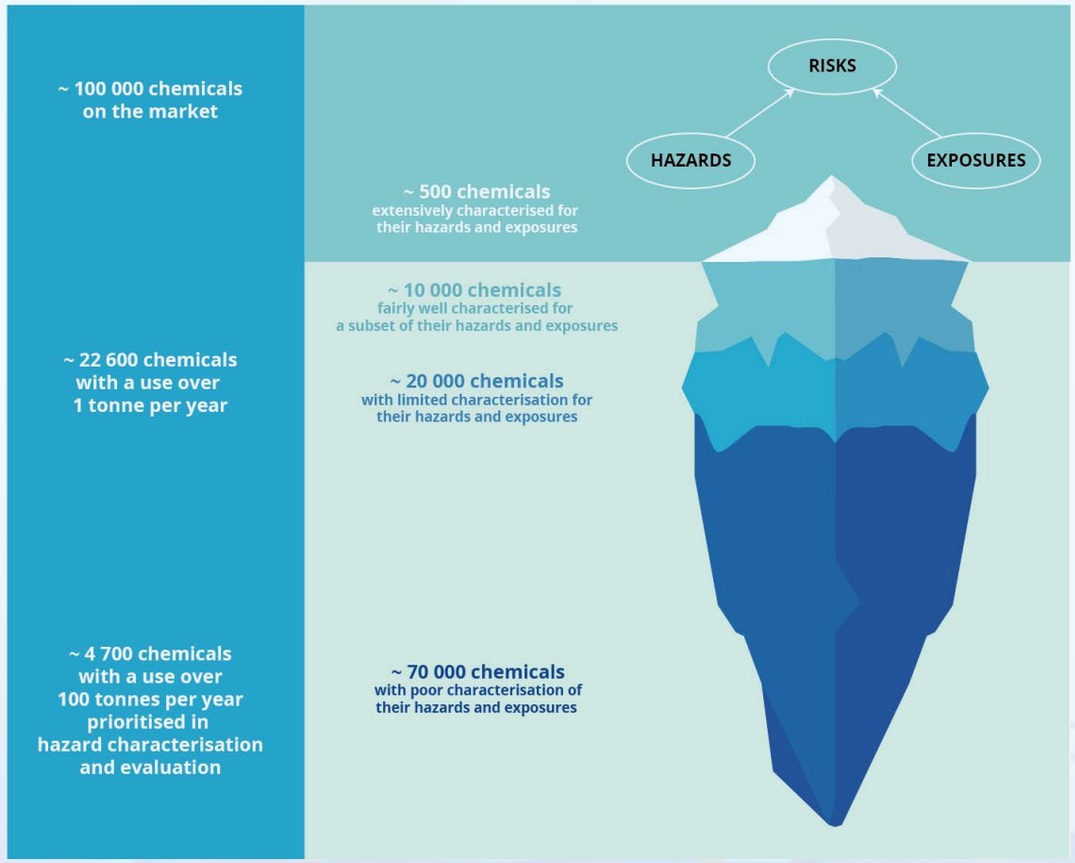
While not defining ‘toxic-free’, the EU Chemicals Strategy does define safe and sustainable-by-design as “a pre-market approach to chemicals that focuses on providing a function (or service), while avoiding volumes and chemical properties that may be harmful to human health or the environment, in particular groups of chemicals likely to be (eco) toxic, persistent, bio-accumulative or mobile. Overall sustainability should be ensured by minimising the environmental footprint of chemicals in particular on climate change, resource use, ecosystems and biodiversity from a lifecycle perspective.”

Despite the lean towards environmental protection, the strategy encompasses human toxicity as mentioned in an earlier European Commission report co-authored by the RIVM titled “Study for the strategy for a non-toxic environment of the 7th Environment Action Programme (EAP)”. In this report, it is mentioned that “the term ‘non-toxic environment’ has not been defined in the 7th EAP. However, ‘environment’ should be considered in its broadest terms to include the natural environment, as well as the human, hence including the ‘technosphere’, i.e. workplaces, indoor environments, cities etc. A non-toxic environment should be understood as an

environment that is free of chemical pollution and of exposures to hazardous chemicals at levels that are harmful to human health and to the environment."[3]

The term 'pollution' is also not defined which adds a flair of enigma to the statement in the EU Chemicals Strategy that references an "upcoming zero pollution action plan" as part of "Europe's zero pollution ambition". If the term pollution refers to limiting the release of hazardous chemicals at levels that would constitute a risk to the environment then this is nothing new. REACH already advocates that risks to human health and the environment must be adequately controlled.

The iceberg of "The unknown territory of chemical risks" as depicted in the EU Chemicals Strategy:



The report goes on to state that "the vast majority of chemicals in the EU is currently regulated on a case-by-case basis and for each specific use. Ample evidence and citizens' worries justify that for the most harmful chemicals the generic approach to risk management becomes the default option, in particular as regards their use in consumer products." This generic approach to risk management has been criticized by Herzler et al. as being hazard-based[2]. To their point, the Chemicals Strategy mentions that the Commission will extend the generic approach to risk management to ensure that consumer products do not contain chemicals that cause cancers, gene mutations, affect the reproductive or the endocrine system, or are persistent and bioaccumulative. Secondly, the Commission says that it will "immediately launch a comprehensive impact assessment to define the modalities and timing for extending the same generic approach, with regard to consumer products, to further chemicals, including those affecting the immune, neurological or respiratory systems and chemicals toxic to a specific organ." This approach does seem to lean heavily on hazards rather than risk.

Another main point of interest is the strategy's aim to tackle the effects of chemical mixtures within risk assessments. One of the underlying principles is what is termed combination effects where "The total risk related to the exposure to a combination of chemicals typically exceeds the risk related to the exposure to each of the individual chemicals in the mixture on their own, at their respective concentration in the mixture."[4] This is yet another point of debate with some questioning the scientific underpinning of such effects[2].

Overall, the call for toxic-free and pollution-free environments, in combination with a greater emphasis on mixture risk assessments is well-intentioned but still needs further refinement in order to get the wider scientific community onboard. ■

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Chemicals Strategy for Sustainability:

what are the practical effects?

By *Berend Mensink, Senior Ecotoxicologist, RoyalHaskoningDHV*

In October 2020, the European Commission presented its Chemicals Strategy for Sustainability. This strategy focuses on protecting citizens and the environment and encouraging innovation towards safe, sustainable chemicals. The Commission links its strategy to the EU's equally ambitious Green Deal, an important re-assessment of our society's future approach to raw materials and substances. Striving for zero pollution, zero waste and zero tolerance is the goal.

Way forward - Safe by design

Society's desire to reuse products and minimise their harm leads to an ever-growing number of new safety requirements. Requirements for the entire production chain, including the design phase: safe by design. There are two ways of ensuring a product's safety. The first way is to avoid using harmful substances. This requires knowing exactly which substances are used; both in the product and during production. The second way is to ensure that substances in the product do not cause harm to people and the environment. Whether the latter happens depends strongly on how the product is used, as using the product does not necessarily equate to exposure to harmful substances.

The focus on reusing and recycling in the European Union's Chemicals Strategy has led to a shift in safety needs and requirements: We accept fewer risks than we used to. Namely, in the past companies used to focus mainly on price. Sometimes, the potential risks of harmful substances were accepted as these substances didn't cause damage when a product was used as intended. Or because people and the environment weren't exposed to a product's harmful substances when using the product as permitted. These arguments are much less relevant when it comes to reusing or recycling products. In those cases, there is a greater risk of exposure which makes it much harder – if not impossible – to recycle materials. As such, the pursuit of circularity further justifies the need to reduce the use of harmful substances in (the design of) the product.

Reducing the use of harmful substances looks easy enough: simply implement a legal ban on the use of a harmful substance. But this is only the first step; monitoring compliance is essential. And monitoring isn't the only thing to bear in mind: you will need to have safe alternatives in place as well. This is where it becomes tricky, because there's often a lack of ready-to-use, non-harmful alternatives. As such, there may be negative social consequences to eliminating harmful substances, besides the obvious positive ones. If a harmful substance is essential to a product and there is no technical or economic alternative, a ban on the substance may also mean a ban on the product – a potentially much more undesirable effect. A good example of this are octylphenol ethoxylates, a group of harmful substances that are crucial to the production of vaccines and medical diagnostics. This example clearly shows the dilemma surrounding this topic.

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It is essential that alternative substances are available, otherwise a ban might not be the preferred solution. This requires research and innovation. But who will be paying for this? For most individual companies, the costs are too high. Innovation shared by several companies in a chain may spread the investment risks, but poses the issue of intellectual property. It also tends to take extensive research and use of the product to determine whether an alternative substance may be carcinogenic or allergenic. Take nanotechnology, for example. This is an area we still know relatively little about and the assessment criteria for safe design and safe use are still in development. Authorities must do more than the prohibition of harmful substances alone. They must contribute to finding non-harmful alternatives by facilitating innovation; funding both fundamental and applied research and bringing innovative parties together.

Does this mean that companies should just wait until a harmful substance has been banned and a safe alternative has been found? Certainly not! The demand for circular products will not lessen any time soon, and neither will society's demand for safe, healthy and eco-friendly products. The general public also has an increasingly positive view of innovative companies with a green image. This means that there's a great risk – a much larger risk, perhaps – in doing nothing. Companies that fail to innovate are less attractive to investors, and it's those investors that are desperately needed for the innovation of substances or production methods. A company that doesn't act and fails to respond to the demand for cleaner and safer products will eventually miss out. It will be left with harmful products that cannot be recycled and that no one wants. ■

PET course
Safe by Design

One of the three topics presented in the first edition of the PET course Current Topics in Toxicology is Safe by Design.

During a 3,5 hour workshop dr. Margriet van der Zee (Sciencelines) and dr. Sam Krouwel (RIVM) will address the basics of creating societal robust and safe innovative products. You will be introduced into the (developing) concepts of Safe-by-Design and Safe Innovation after which an interactive Serious Game will be played, revolving around a fictive case of an innovative product. At the end of the workshop, you have learnt what potential risks need to be considered and how they can be addressed at an early stage of product research and development.

In addition to Safe by Design, also the topics New Developments in Human Based Models and Endocrine Disruptors will be presented in the course. Please check https://www.toxcourses.nl/courses/current-topics-in-toxicology/ for more information and to register. ■

Course coordinators: prof. dr. Juliette Legler and prof. dr. Flemming Cassee
Intended for: (registered) toxicologists
Date: 2-3 December 2021
Credits: 3 CPD credits (Continuing Professional Development)
Location: Congress Centrum Woudschoten, Zeist

Case study:

Safe by Design to avoid a regrettable substitution

By *Héloïse Proquin*

Safe by design is an important concept when developing new compounds but also to avoid the potential regrettable substitution problem. A regrettable substitution is when the alternative substance has similar or even more toxic effects compared to the banned one.

An example of regrettable substitution is the case of Bisphenol A. Bisphenol A (BPA) is used in combination with other chemicals to manufacture plastics and resins. BPA is also used in a number of non food-related applications, including epoxy-resin based paints, medical devices, dental sealants, surface coatings, printing inks and flame retardants[1]. Because new data was published, EFSA decided, in 2012, to re-evaluate BPA. According to ECHA, BPA is now considered as a substance that "may damage fertility and has been identified as a substance affecting the hormonal systems of humans and animals. In addition, it damages eyes and may cause allergic skin reactions and respiratory irritation."[2]. Therefore, it is now prohibited in baby-bottles[3], in infant drinking cups[4], in cosmetics[5], and restricted in thermal paper[6].

The public concern and restrictions on BPA stimulated the development and production of alternative substances or alternative products to replace BPA in many applications. A review made by den Braver-Sewradj et al. showed a total of 99 alternative substances[7]. A selection of 20 for full assessment was performed depending on the use by the general population. In general, data gaps were found for most of these substances. Within these 20 alternative substances, 5 (Tefacid Stearic 95, Bisphenol C, AP, and P) had limited or no information at all on endocrine disrupting potential, carcinogenicity, and reproductive toxicity. Data was available - indicating a reproductive toxicity hazard with a possible endocrine disrupting mode of action - for bisphenol S (BPS), bisphenol AF (BPAF), p-tert-butylphenol and to a lesser extent bisphenol F (BPF), fluorine-9-bisphenol (BHPF), bisphenol E, M, and Z (BPE, BPM, BPZ), Irganox 1076, and butylated hydroxytoluene (BHT). Positive studies for carcinogenicity were found for 3,3□,5,5□-Tetrabromobisphenol A (TBBPA). Negative data on reproductive toxicity and/or endocrine disrupting potential were found for benzoic acid and Irganox 1010, tetra methyl bis phenol F (TMBPF), and bisphenol-A bis(diphenyl phosphate) (BDP). However, these data were not complete. Another study concluded that BPS, BPG and BPF were having similar properties as BPA and might induce side effects at lower doses[8]. Taken the above, some alternative substances to replace BPA can be considered regrettable substitutes.

This short case study is an example of the importance of evaluating the alternatives in parallel to banning the use of a substance: sufficient information should be available for alternatives in order to avoid regrettable substitution. As a full hazard assessment including reproductive toxicity studies and carcinogenicity studies is not possible for all potential alternative substances, SbD approaches could be used. SbD aims to predict potential human and environmental hazard early in product development. In addition, SbD focuses on physicochemical properties that influence toxicity and provides users options to make their product safer. For the case of BPA, alternatives could be compared to BPA using *in silico* and *in vitro* methods. Those alternatives that show lower potency to induce ED could be selected for further assessment. In this way, resources can be saved to test alternatives that have a higher chance of being safer compared to the original substance. Important to note is that SbD approaches are still under development and that a validated *in silico* and *in vitro* testing strategy to predict ED potential is lacking. To avoid regrettable substitution, there is need for clear SbD strategies that rely on predictive methods. ■

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A review of the Herzler et al. critical paper on the EU Chemicals Strategy for Sustainability

By *Damiën van Berlo*

In June of this year, an article was published in Archives of Toxicology by the German Federal Institute for Risk Assessment (BfR) titled "The 'EU chemicals strategy for sustainability' questions regulatory toxicology as we know it: is it all rooted in sound scientific evidence?" (*Herzler et al., 2021*). The BfR is not the only German organization involved in the regulation of chemicals: The Federal Institute for Occupational Safety and Health (BAuA) and the German Environment Agency (UBA) are two other German institutes that contribute to, and are responsible for, chemical regulation under REACH. The article critically reflected on some aspects of the EU Chemicals Strategy for Sustainability (CSS; published on the 14th of October 2020), which encompasses the vision of the European Commission on a safer, less polluted environment. In particular, the authors questioned the scientific justification of the zero pollution ambition for a toxic-free environment and the proposed approach to risk management for different groups of hazardous chemicals and mixtures.

The author is an employee of Bureau REACH, which is embedded within the RIVM and is delegated by the Dutch government to carry out the member state duties related to the the REACH- and CLP Regulations. Disclaimer: the views expressed in the current article belong to the author and not Bureau REACH, its affiliates, or employees.

A number of interesting points are raised by the authors, however, the arguments that are brought forward will likely not universally be accepted and have sparked discussion among regulatory toxicologists. When considering the Herzler et al. paper as a whole, it is to be noted that the authors included only consumer issues in their assessment; the potential impact for workers and environment were not taken into consideration, while these impacts were included in the CSS. Some of the main points are summarized below, along with a critical assessment of each of these points, with the aim to give the TCDD reader an impression of the discussions centered around the CSS and the Herzler et al. (2021) paper. This piece should, therefore, be considered as a sample rather than a complete presentation of the argumentation from all sides. The main focus of this piece will be on aspects that are relevant within the context of the REACH legislation for chemicals. Aspects that influence other regulations that are affected by the CSS, such as legislation for biocides, cosmetics and food contact materials, are not specifically addressed. The main arguments that are brought forward by Herzler et al. are briefly summarized, followed by the author's reflection; emphasis by underscore is added by the author.

1. The authors of the Herzler et al. paper argue that the ambitious plans to implement changes in chemicals legislation proposed in the CSS suggest that the current system of chemicals legislation is ineffective, since, in their opinion, a very high level of protection and environmental quality has already been achieved in the EU. According to Herzler et al. (2021), this is confirmed by a high life expectancy and growth rate of the European population, which does not indicate that there might be a fundamental problem with regard to reproduction. The authors conclude that the CSS' justification of the action needed is based on public concern, not scientific argumentation;

A desire to improve and adapt to emerging risks and new insights is a crucial state of mind that is needed to properly regulate chemical substances. Legislation such as REACH is never a finished product: it should be re-evaluated on a regular basis and amended if deemed appropriate. For instance, for the regulation of nanomaterials the initial REACH legislation was not sufficiently suitable for this new scientific and technical development and it has been adapted to accommodate regulation of this particular class of substances. The ambition to further improve legislation that at its core has provided enormous benefits to public

health and environmental quality, should be considered as a sensible thing to do. For this reason, reviews of the REACH Regulations have already been carried out in the past. Furthermore, the suggestion that chemical reproductive risks are sufficiently controlled based on observed historical trends for life expectancy and population in the EU is scientifically unconvincing. Several important factors are known to have contributed to these beneficial trends, including improved nutrition and healthcare; such major factors can easily mask a smaller effect on reproductive health due to chemical exposure. This would not mean that these potential harmful effects are not important or relevant.

2. Herzler et al. (2021) argue that the CSS intends to improve efficiency of the existing system, while not recognizing that delays are most often due to a lack of crucial information needed to perform risk assessment and the bureaucratic nature of the processes aimed at obtaining missing/additional information; non-compliance by the industry is one of the biggest obstacles for identifying substance of concern under REACH;

As the authors correctly point out, the time between the recognition of a chemical health threat and the final regulation (time-to-regulation) of this particular substance (for instance by restricting its use or by classification and labelling) is far too long; in the meantime, the substance is released into the environment and people are continued to be put at risk. Indeed, the availability of test results of sufficient quality for risk assessment remains a challenge. For low-tonnage substances for example, information requirements are very limited, which may for certain uses be a concern as low tonnage does not necessarily mean low exposure or limited potential health impact . This means that we know very little about the potential hazards of these chemicals. It is possible to request more information, but streamlining these processes is not easy. Member State Competent Authorities (MSCAs; a national authority responsible for the implementation of REACH) actively participate in discussions with the European Chemicals Agency (ECHA) to accelerate processes such as Dossier Evaluation and Substance Evaluation. In fact, it may be legal issues rather than bureaucratic issues that can significantly impair progress. It is known that registrants (i.e., the industry) have multiple opportunities to intervene during the process which causes delays. When they do not agree with a decision, they can bring their case to the ECHA Board of Appeal (BoA), which usually results in a multiple-year delay. Moreover, cases can be brought to the European Court which delays the process even further. As of yet, there is no consensus on how this problem might be addressed.

3. Herzler et al. (2021) criticize that the "zero pollution ambition for a toxic-free environment", being a core ambition of the CSS, is poorly defined and erroneously quoted from other documents. Furthermore, it is argued that, in the current formulation, the zero-pollution goal is not realistically achievable because, ultimately, any chemical can lead to toxic effects depending on the dose ("it is the dose that makes the poison"). Moreover, the original interpretation of this goal is highly similar to the existing REACH paradigm of an "adequately controlled risk".

This is a fair criticism; when the main aim of the CSS is to achieve a "toxic-free environment", it needs to be defined what exactly 'toxic-free' means in practice. Especially because at first glance, it is immediately apparent to any toxicologist that a toxic-free environment, when this is interpreted literally, is not a viable scenario. The original wording in the report from Goldenman et al., in which the new strategy was explored, is to strive for an environment that is "…free of chemical pollution and of exposures to hazardous chemicals at levels that are harmful to human health and to the environment." (*Goldenman et al., 2017*).

4. Herzler et al. (2021) argue that, in contrast to what is suggested in the CSS, chemicals should not be regulated based on their hazard, but based on risk (i.e., exposure should be taken into account); for instance, personal computers, mobile phones and cars contain hazardous chemicals but nevertheless should not be considered to pose a threat;

In a perfect world, no toxicologist could disagree with chemical regulation based on risk instead of hazard. However, in the current world we will have to make do with a reality where the availability of information is very limited and data on exposure for many chemicals are virtually non-existent. Moreover, the uses of these chemicals, and thus exposure throughout life cycle, changes continuously whereas the intrinsic properties of substances can also vary depending on the life cycle stage. So as logical and scientifically sound as it may seem, in many cases it is simply not possible to conduct detailed risk assessment for all uses and all populations, while keeping track of all changes regarding use. Setting priorities on intrinsic hazard is an efficient first step for further risk management.

5. According to the authors the precautionary principle should be used responsibly. In their interpretation it should be applied only when sufficient scientific arguments for an existing risk are available, quote: "In contrast …, the precautionary principle is now frequently called upon in the absence of demonstrated risks."; setting criteria for deciding on the adequacy, proportionality, and commensurability of such measures will be a key task for the European Commission;

This argument is based on the idea that a risk needs to be demonstrated before regulatory action is to be considered. Firstly, one can question whether regulatory action would still be considered "precautionary" after a risk is demonstrated; so in fact the authors appear to state that they are not against the precautionary principle, but that it should not be used in a precautionary manner. This is a confusing opinion. It is clear however, that one should certainly avoid unnecessary withdrawal of substances from the market as they usually have clear functional and social benefits (otherwise there would be no market). From a public health perspective however, it makes a lot more sense to bring a substance to the market after it has been shown to be safe (i.e. fulfills the criteria that define whether a chemical is considered "safe" under the conditions of use), than to withdraw it after it has been shown to be a threat to public health or the environment. The challenge will be, in agreement with what the authors state on this, to define when regulatory action should be taken in the absence of reliable data. It might be reasonable to use the precautionary principle in order to lay the burden of proof on the side of the registrant. This would imply that a chemical should be withdrawn from the market unless the registrant demonstrates the safety of its uses with sufficient data. In principle, this is already a leading proposition of the REACH Registration obligation (no data no market principle), although in specific situations (e.g., for low tonnage substances) only limited information needs to be provided before introduction onto the market.

6. According to the authors, the CSS implicitly claims that exposure to chemical mixtures constitutes a major and generic health problem requiring immediate regulatory action; evidence to support this claim is lacking and a generic Mixture Assessment Factor should not be applied;

The assessment of mixture exposure and their effects is notoriously difficult; epidemiological studies can give an idea about correlation, but causation is much more difficult. Outside of disasters, where people are accidentally exposed to harmful doses of a specific chemical, solid data in humans that shown causal effects are difficult to come by. Emphasis should be placed on the fact that these difficulties exist when assessing single substances; the situation becomes much more complex when considering unintentional mixtures. The large numbers of chemicals in such mixtures to which humans are exposed at various locations and points in time make it very challenging to "map" human exposure, let alone attribute health effects to it. However, there is evidence for mixture effects in (freshwater) aquatic ecosystems, on the population level (Kortenkamp et al., 2019); we can consider (populations of) animals as models for (populations of) humans, i.e., it is reasonable to assume that adverse effects will be seen in humans as well. Being prudent is probably a wiser choice than waiting for solid evidence in humans, which will mean that mixture exposure will already have occurred in the population.

7. Finally, Herzler et al. (2021) contradict the argument made in the CSS that endocrine disruptor (ED)-related risks are not sufficiently mitigated by the current regulatory system; they argue that this statement is poorly founded with scientific data; also, most ED substances are already regulated because of their carcinogenicity or reproductive toxicity;

It is true that several currently identified ED substances induce effects that warrant classification in other CLP hazard classes. However, the impact of those classifications may be limited as, for example, a STOT-RE classification does not normally trigger regulatory measures comparable to identified ED substances. The focus on ED substances has partly resulted from the long-lasting discussion about whether such substances have a threshold or not. Given the complexity of the scientific discussions it is imaginable that no consensus will be reached, but when considering the precautionary principle, it would be preferable to take action on such substances rather than wait until a final conclusion is reached. ■

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AIO toxafette - Nienke Ruijter

By *Nienke Ruijter, PhD Student Nanotoxicology at RIVM National Institute for Public Health and the Environment.*

1. Can you introduce yourself?
Hi, I'm Nienke, and I'm in the second year of my PhD-research at the RIVM in Bilthoven. I'm from Wageningen, and also did my bachelors and masters there. My hobby is slalom kayaking, in which I also like to compete.

2. How would you explain the subject of your research project to a layperson?
My PhD is part of a big EU project, called SAbyNA. Within the project, we're making a web-based platform that allows people to get an idea of the safety of a nanomaterial, or a product containing nanomaterials. The platform will be made especially for manufacturers of these products, to help them assess the safety of their product already during the early stages of product development. When going through the process, the platform may recommend performing some simple laboratory tests. This is the part that I'm working on in my PhD. I am assessing the suitability of already existing *in vitro* tests for this strategy, and optimizing them to become more suitable. In the end, the goal is to put together a test-battery that is perfectly tailored for nanomaterials and for the assessment of hazards already during the early stages of product development.

3. What was your motivation to start a PhD-program?
I was certain that I wanted to do a PhD-program when I was doing my master thesis and internship. Research is just really my thing! Not very surprising, since I come from a family full of scientists. After finishing my masters, I first took some time off to travel. When I returned, the motivation for doing a PhD-project had kind of dropped, and I started looking for other jobs first. But during my job search, it was always the PhD positions that interested me the most.

4. Why did you choose a subject in toxicology?
I studied nutrition and toxicology in Wageningen, so toxicology was already a topic of interest for me. However, during my master thesis and internship, I went more towards the molecular biology side, figuring out molecular mechanisms at a very detailed level. This PhD is very different from that, but has a much bigger direct impact. That's one of the things that really attracted me about doing this PhD-research in toxicology.

5. How do you see the future of your research topic (follow-up research / social impact)? What do you hope for?
The two main themes in my research are 'animal-free testing' and 'safe-by-design'. I hope to contribute to reducing the number of animals used for toxicological testing, by optimizing *in vitro* methods. I also hope that the concept of safe-by-design will be applied more in industry, and that the SAbyNA platform can encourage manufacturers to pay more attention to product safety.

6. What is the best advice that you have received as a PhD student or would like to give to another PhD student?
A little while ago, I took a course in academic writing with Artesc, and they gave some advice that I think would be relevant for a lot of PhD students, namely: 'You are not a writer, you are merely a creator of new versions.' Thinking about this really takes the pressure off from writing articles. It doesn't have to be perfect!

7. Are you a member of a society and what do you expect from being a member?
I am a member of Proneri, the PhD-student network at RIVM. And I am a member of the NVT, where I am now in the committee to organize the NVT meeting of 2022. I hope to expand my network, and have a lot of fun doing this. I hope to see you all at the NVT meeting next year!

8. How do you combine your PhD project with your personal life? Are there choices you have to make?
I started my PhD-project during COVID, so I didn't have much of a personal life going on. There weren't any parties or trips or anything, so it was easy to combine (and easy to become a work-a-holic). Now that things are loosening up again, I have to find a new balance. What I notice now is that I am the person who is always late for things, because I'm in the lab until late, but I guess that's alright.

9. Does the project meet your expectations, why or why not?

I really like my PhD-research. The thing that I like most about it, is that it is part of this larger overarching project. I work together with people from institutes from all over Europe, which gives me a lot of energy. Of course, I also have long, and sometimes frustrating days in the lab, but there is more variety to my PhD than just that. Normally (without COVID), my PhD would also consist of some traveling to other institutes. I'm really looking forward to that finally happening.

10. What goals do you have regarding your career after your PhD? Would this be inside or outside academia, and why? Would you consider going abroad?

After finalizing my PhD, I would like to do a post-doc abroad. I love the mountains and mountain sports, and I kind of miss that in the Netherlands. Doing a post-doc somewhere in or near the Alps, or another mountain range, would be amazing.

11. Please answer the question from the last toxafette PhD-candidate: "What do you expect from your PhD on a personal level, how will the experience change you?"

On a personal level, I hope to gain more confidence during my PhD-project. I have the natural tendency to stick to the background and observe. I have now been given some coordinating roles within my project, and I think this will really help me develop some crucial skills. ■

Registratie Cie

TiO's:

Voorletters	Achternaam	Opleider	Datum inschrijving
C.	Henstra	Prof.dr. D.J. Touw	23/06/2021
D.H.	Swart	Prof.dr. D.J. Touw	23/06/2021
H.	Brouwer	Prof.dr.ir. I.M.C.M. Rietjens	23/06/2021
W.B.	de Beer	Prof.dr.ir. I.M.C.M. Rietjens	23/06/2021
F.	Akuamoa	Prof.dr.ir. I.M.C.M. Rietjens	13/09/2021
K.S.	Nitsche	Prof.dr.ir. I.M.C.M. Rietjens	13/09/2021
E.J.	Streekstra	Prof.dr. F.G.M. Russel	13/09/2021
X.	Zhang	Prof.dr.ir. I.M.C.M. Rietjens	13/09/2021
K.E.K.	Maudens	Prof.dr. F.G.M. Russel	13/09/2021

Registrants:

Voorletters	Achternaam	Datum inschrijving	Datum afloop registratie
I.E.	Janssen	26/03/2021	26/03/2026
C.	Bethlehem	26/03/2021	26/03/2026
Y.	Wei	13/09/2021	13/09/2026
I.	Gilbert Sandoval	13/09/2021	13/09/2026
A.	Koppen	13/09/2021	13/09/2026
G.A.M.	Eliesen	13/09/2021	13/09/2026
C.H.C.	Litjens	13/09/2021	13/09/2026
V.C.	de Leeuw	13/09/2021	13/09/2026

Beyond gene expression:

novel methods and applications of transcript expression analyses in RNA-Seq

By *Rajinder Gupta*

I joined the department of Toxicogenomics, FHML, Maastricht University in May 2016 under the EU-ToxRisk project – An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st-century. My supervisors were Prof. Dr. Jos Kleinjans and Dr. Florian Caiment. I defended my thesis 'Beyond gene expression: novel methods and applications of transcript expression analyses in RNA-Seq' via a zoom call on 3rd March 2021, from my apartment in Freising, Germany. Had the times been better, my family, friends, and colleagues would have joined me in person and we would have celebrated the occasion. A few days later, the PhD-office sent the diploma by post and all the hard work for the last four and a half years felt nothing less than a dream come true.

Hypothesis

Gene expression is heterogeneous and expression profiling using traditional methods, such as quantitative PCR, can mask the changes that occur at the transcript level. The analysis of transcript expression, i.e. the set of all RNA transcripts, including coding and non-coding transcripts, is more informative than analysis performed only protein coding transcripts.

Motivation

Transcriptomics, the study of the transcriptome, provides a more comprehensive view of gene expression. Gene expression results in protein-coding and non-coding transcripts originating from a given gene, this makes gene expression heterogenous. The precise quantification of transcripts has been made possible with the increased sequencing depth, computational power, and is now possible at comparatively lower costs.

Challenges

While the study of transcript expression holds the hope for a better understanding of the biological processes, it also poses a few challenges. Firstly, the available biological annotation databases are gene/protein-centric and hence inferences cannot be directly derived from the transcript-based results. Another major challenge is that various protein-coding transcripts originating from the same or different genes can code for proteins that perform similar/same functions. So, analyzing each transcript individually would fail to reflect the functional changes in the biological system.

Methods/Experimental Design and Results

We compared healthy human liver tissue with *in vitro* liver cell lines and, from the transcriptomics data, computed the differentially expressed and non-differentially expressed genes (DEGs and non-DEGs). To supplement these findings with the transcript level changes, differential transcript usage per gene was evaluated. The genes for which any transcript exhibited differential usage, were removed from the list of the non-DEGs (non-DEGs^{DTU-}) (Figure 1). The final list of genes generated to capture the changes at the gene and transcript level and provides the gene identifiers to be mapped to biological databases for functional annotation.

The non-DEGs that exhibited differential transcript usage (DTU) show that many changes occur at the transcript expression level, however, are not captured at the gene expression level. We tested our approach using data from various human *in vitro* liver cell models compared to healthy human liver biopsies. We found that from the non-DEGs, the most expressed protein-coding transcript in healthy liver biopsies is less or not expressed at all in the *in vitro* cell models. Under gene expression-based

analysis, these genes would be categorized as non-DEGs, hence unperturbed however with the inclusion of DTU, perturbations for these genes are brought to light.

Furthermore, through alternative splicing (AS), genes can produce various transcripts. The protein-coding transcripts from the same or different genes can code for the same/similar functions. To identify such protein-coding transcripts, their amino acid sequences, secondary and super-secondary structures, and protein families were compared. It was seen that various protein-coding transcripts originating from the same gene or genes from the same gene family shared functional similarity. To our surprise transcripts from unrelated genes also exhibited functional similarity, however they were not the longest protein-coding transcript from the gene. The similar function proteins were grouped to form the SFPGs (similar function protein groups). The methodology to compare and group the transcripts based on their functions is developed as a tool namely, FuSe, that can be assessed at https://github.com/rajinder4489/FuSe.

The application of FuSe on the liver cell models, treated with therapeutic and toxic APAP (acetaminophen) doses revealed that the expression patterns of the individual transcripts vary from the functional groups (SFPGs) . The new findings align with our understanding of APAP action and its effects on the body.

Conclusion

The differential expression and/or usage of the transcripts hold important biological signals. These changes are a result of AS; however, gene expression-based analysis fails to capture these signals. Due to the unavailability of transcript-centric biological annotations, we adapted an innovative approach to quantify non-DEGs^{DTU-}, that grasped the changes at the transcript level and connected the results to the gene/protein annotations in the biological databases. The protein-coding transcripts from the same or different genes can code for similar function proteins. Grouping these functionally similar transcripts allows for functional analysis of the transcriptomics data and generating functionally relevant results. An approach is developed to identify, and group these transcripts and plug them into the transcriptomics data analysis pipeline. The use of transcript expression provides biologically relevant results. The changes in the fractions of the transcripts produced (protein-coding or non-coding) can result in up- or down-regulation of many downstream processes. The assessment of expression pattern changes using SFPGs reflects the functional changes with precision.

Thesis available at:

https://doi.org/10.26481/dis.20210304rg

Related publications:

Gupta, Rajinder, et al. “Comparing *in vitro* human liver models to *in vivo* human liver using RNA-Seq.” Archives of toxicology 95.2 (2021): 573-589.

Gupta, Rajinder, et al. “FuSe: A tool to move RNA-Seq analyses from chromosomal/gene loci to functional grouping of mRNA transcripts.” Bioinformatics 1 (2020): 7.

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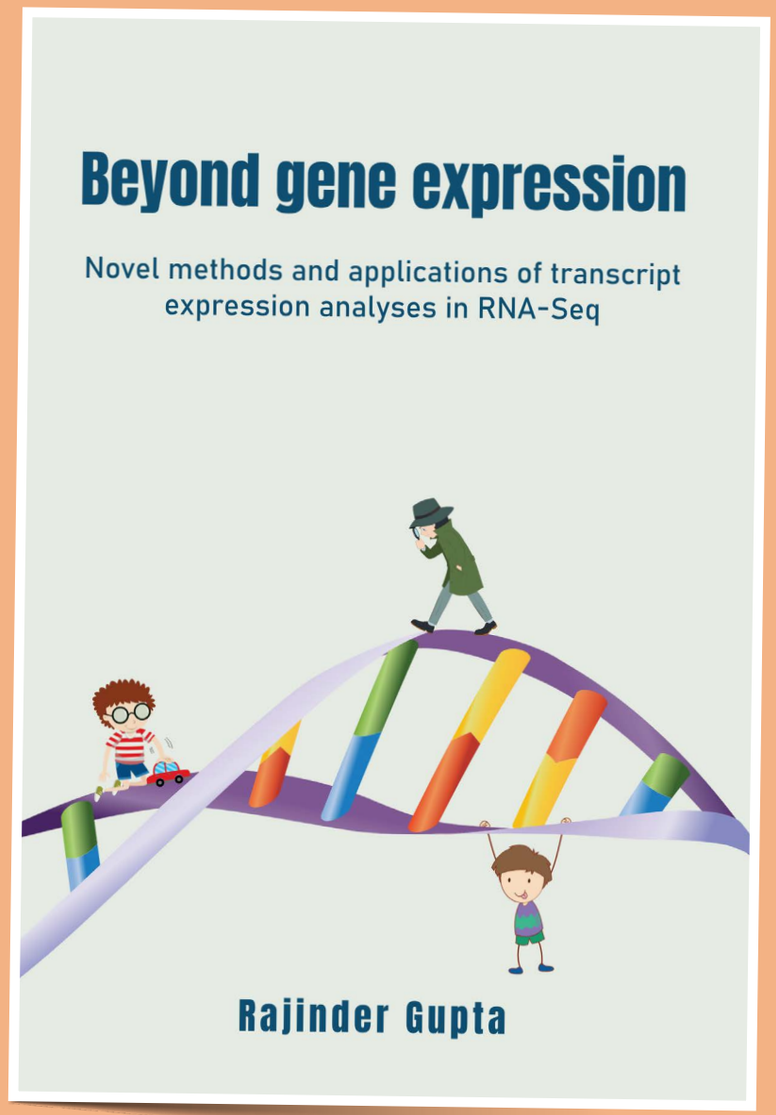

Skin Sensitization:

a new, OECD adopted, *in vitro* testing strategy and add-ons to take into account potency

By *Bennard van Ravenzwaay, Susanne N. Kolle, Robert Landsiedel. BASF SE, Experimental Toxicology and Ecology. Ludwigshafen, Germany*

„Gut Ding braucht Weile“ – a German expression which may be translated as “a good thing takes its time”, while this is generally true, it appears to be particularly applicable to the regulatory acceptance of alternative methods. Before a new product is approved by the authorities and placed on the market, numerous tests must be carried out. This includes testing whether the product causes skin or eye irritation as well as if it sensitizes the skin. In the past this has required animal testing. The development of alternative methods to address topical (skin, eye) irritation started decades ago. Many methods were developed; many were rejected later and only a few methods were eventually validated and accepted for regulatory use. Validation for regulatory purposes is a long, though, uphill battle that usually takes 10 years or more. To assess potential skin and eye irritation *in vitro* several methods are available and at least two methods need to be combined to provide the information necessary for classification and labelling (C&L) purposes. This was the first indication that for replacement of animal testing, even for relatively simple end points of toxicity, a testing strategy is necessary.

Compared to skin irritation, skin sensitization is a far more complex process of chemico – biological interactions. In Fig. 1 the necessary sequence of events, also referred to as the adverse outcome pathway (AOP, (OECD 2012)), is shown. The first step is dermal penetration of the compound. This is followed by the so called molecular initiating event (MIE) which is the chemical reaction of the compound with the skin protein. One of the cellular events following is the activation of keratinocytes inducing an alarm signal. The next step in the AOP is the recognition of these altered cells by the immune system, in the skin represented by dendritic cells, thus initiating the first local immune response. The dendritic cells change their surface structure then migrate to the lymph nodes, presenting the antigen and inducing a systemic immune response. Upon subsequent exposure to the same allergen, a skin inflammation reaction is elicited.

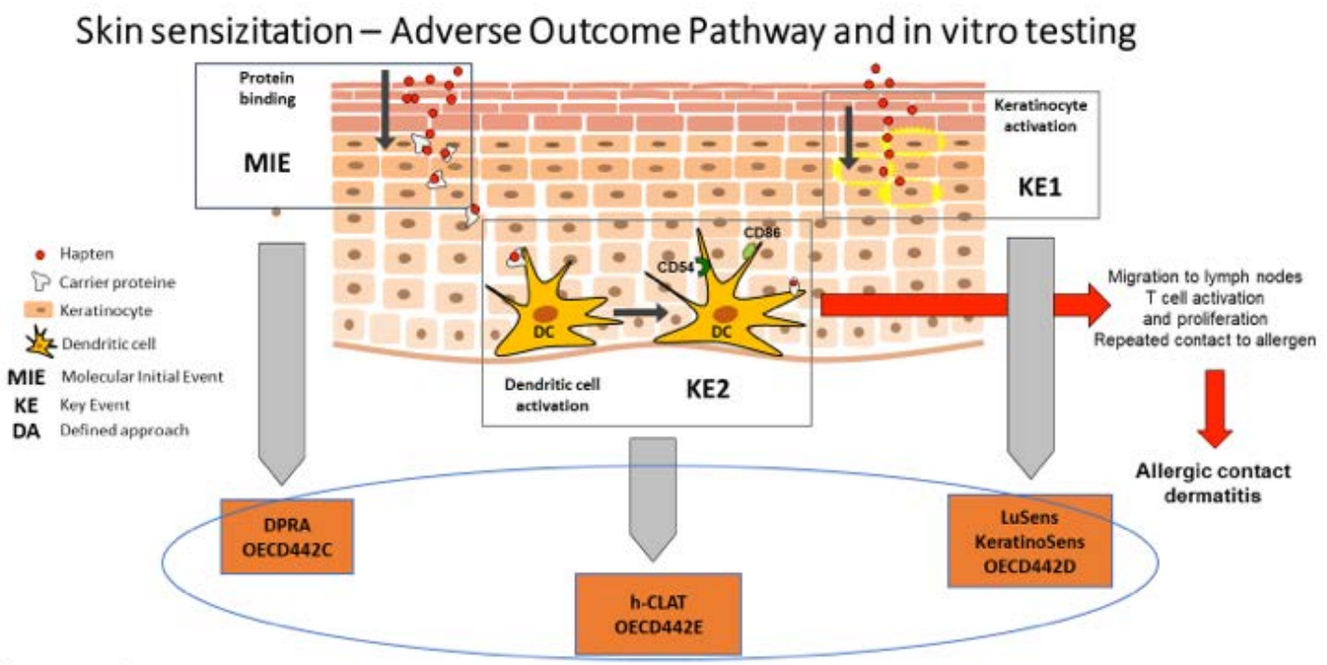

Fig 1: *The Adverse outcome Pathway for skin sensitization and selected non-animal methods available to assess this AOP.*

Taken into account the complexity of this chemico – biological interaction and the many cellular players involved, it is not necessarily a surprise that such an *in vivo* process cannot be replaced by a single alternative method. To address this need, several *in vitro* methods were developed which are placed at key events along the AOP for skin sensitization. These include (1) chemical reactivity with proteins (mimicking the MIE), (2) the cellular stress response of the skin cells and (3) the response of the dendritic cells (the cellular immune response). Like for the less complex questions related to skin irritation, here too, a testing strategy is necessary to combine the results of the individual methods to predict whether or not a substance is a sensitizer.

Recently, and again after many years of validation and meetings to convince the regulatory community, the world's first toxicology testing strategy without animal testing has been approved by the OECD (Organisation for Economic Co-operation and Development) as OECD guideline 497 (OECD 2021a). The "2 out of 3" testing strategy consists of the above mentioned three alternative methods, addressing the key points in the skin sensitization AOP. They can be used to predict whether a substance causes skin sensitization. Unlike in the past, animal testing will no longer be necessary for this end point for defined organic substances that can be tested in an *in vitro* setup. This testing strategy was developed and validated in a joint effort by BASF, Givaudan, P&G, Kao and Shiseido (Urbisch et al. 2015). It does not only provide a way to investigate skin allergy *in vitro*, which is at least as predictive of human data as the animal testing, it is also the first time that the concept itself (developing alternative methods to investigate key elements of the AOP) is adopted by the OECD. This will be the way to develop and use alternative methods to answer more complex toxicological questions without animal testing.

Unfortunately, due to the demand by C&L, it is not enough to assess if a compound is a skin sensitizer or not. The potency of the allergen must also be determined. Therefore, even after the establishment of the "2 out of 3" -test strategy further research was needed to see if one or more of these methods could be used to determine the intensity of the allergenic response. This further challenge has now been met by enhancing the test related to chemical reactivity (the MIE test) to become the Kinetic Direct Peptide Reactivity Assay (kDPRA (Natsch et al. 2020; Wareing et al. 2020)). This alternative testing method is a further achievement of the joint effort between Givaudan and BASF and has received OECD adoption as updated of OECD test guideline 442C (OECD 2021b). It can complement the approved testing strategy. Only with this additional test can animal testing for allergic reactions now be completely abandoned. Although the two companies have been an important driving force, they haven't done it all alone, of course. Over the past 10 years, various companies and scientific institutions, such as the Institute for *In Vitro* Sciences (IIVS), have worked together to validate the individual methods of the strategy.

Although it is appropriate to celebrate this success, a few words of caution need to be expressed. The requirement to develop animal-free approaches that fulfill the currents requirements for C&L bears a significant jeopardy. The specifications related to specific classifications, e.g. weak, moderate or strong sensitizer, were developed based on *in vivo* data and have been subject to changes over the last decades. So, when alternative methods are developed to address the current requirements (such as the kinetic DPRA), this means that with any change in criteria, or the addition of sub-classifications, the entire testing strategy must be re-adjusted. This clearly bears the risk of freezing the entire current system for years. New adjustment to the criteria may be avoided, even though they might be appropriate, because it would require different / adjusted methods (which take some time as shown above), or, new alternative approaches are not developed anymore because they have to be appropriate for current C&L requirements, which are clearly designed based *in vivo* data and are not necessarily fit for *in vitro* data. ■

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A poisonous summer

Did you have a good summer? A question which is asked frequently during this time of year. Well, actually we had a good one, especially from a toxicology-in-the-news perspective. We have enjoyed gardening, we got in touch with nature, and enjoyed good food. All with their own stories and poisons. Let's catch up!

By *Carolien Schophuizen*

To eat or not to eat

Homegrown vegetables - On the 4th or April, the parliament was informed about the state of affairs regarding the exposure of people to PFAS via food, drinking water and consumer products[1]. In that letter, sent by the ministers, the advice given by the RIVM to no longer to consume fruit and vegetables from vegetable gardens within a radius of 1 kilometer from the Chemours factory was highlighted. Since 2018, the RIVM advised to consume homegrown food from that area only in moderation, but this has now been adjusted. The reason is that the European health-based limit values for PFAS in food have become stricter[2]. On this basis, the RIVM advises to: no longer consume food from vegetable gardens within a radius of 1 km around the factory Dupont/Chemours and for the allotment complex Sluisdijk[3]. For vegetable gardens further from these locations, the data collected in 2018 provide insufficient clarity. That is why RIVM advised to conduct further research to a wider area around these companies. Until then, as a precautionary measure, the advice is to moderate the intake of homegrown food in the area studied at 1-4 kilometers from the factory.

A farmers' pain

Jacobaea vulgaris, syn. *Senecio jacobaea* - Due to the altered mowing policy (ecological roadside management[4]) in the Netherlands, in combination with the rainy summer of 2021, the roadsides came to full bloom this year. Among the cornucopia of flowering herbs, an abundance of ragwort (Jacobskruiskruid) can be seen. For some time now, the cheerful yellow flowers of the ragwort have been a pain for cattle farmers, in large doses it is poisonous to grazing livestock. Especially when the herb ends up in hay used for feeding, animals may suffer its effects. The Farmers Defence Force is lobbying for a change in mowing policy[5], to avoid the blooming of the herb and thereby prevent the spreading of its seeds.

Animals usually avoid the plant when grazing. The herb is full of pyrrolizidine alkaloids: PAs. PAs have a very bitter taste, and grazers only eat it in extreme distress or when it is mixed into hay. Humans usually do not ingest the plant or parts of the plant, though PA's may end up in honey. The NVWA analyzed honey samples for PAs in 2016, concluding that the average PA content in their sampled honey is 3.4 µg/kg, a level at which even enthusiasts with excessive honey consumption are not at risk[6] (based on an advised maximum PA intake of 0.1 µg/kg bw/day, for non-carcinogenic effects and 0.43 ng/kg bw/day based on possible carcinogenic effects[7]).

Whether a ban on, or an extermination obligation for, ragwort would be necessary is still being debated. Good grassland management prevents cattle from coming into contact with ragwort and the plant often disappears on its own after a few years.

Favourable winds?

Phosphine - This summer, the so-called poison boats (gifboten) have also made the news a number of times. In Veghel, Zwolle and Utrecht barges containing grain with too high a concentration of the fumigant phosphine were detained[8]. Phosphine is added to cargo in pellet or granular form[9] (e.g.

aluminium phosphide or magnesium phosphide granules). When these pellets come into contact with water from the ambient air, phosphine is released by hydrolysis of the metal phosphide.

A criminal investigation has been initiated against the company that withheld information regarding the fumigated cargo of wheat from the three chartered inland shipping skippers[10]. Furthermore, ILT and NVWA (food safety) are currently mapping vulnerabilities in the transport chain[10]. In addition, the Dutch government and the Board for the Authorization of Plant Protection Products and Biocides are advocating a stricter and harmonized application manual for phosphine-producing agents in batches at European level. Not only did the phosphine incidents raise questions regarding the safety of the bargemen and their families living on board, it also stirred a more generalized discussion regarding degassing[11]. Ships carrying hazardous liquid substances sometimes need to degas their holds in order to ship a new load, or another liquid. However, vapours are left behind after unloading. With a fan, air is blown through the tank to clean it, and the vapours dissipate into the open air. Since 2019 this is no longer allowed in densely populated areas, and in Germany as well as Belgium it is already prohibited[12]. As not all shipping terminals are equipped with an expensive degassing installation, many ships are degassing in the open air in the areas where it is still allowed[13], sometimes leading to odor nuisance and health and safety concerns. In the Netherlands, the ban on degassing[14] will be rolled out in 3 phases: 2021: Ban on benzene and motor fuels. (These are the most commonly transported substances in inland shipping); 2023: ban on benzene-like liquids; 2024: ban on remaining most commonly transported substances (regardless of benzene content).

Total recall

Ethylene oxide - Furthermore, there was plenty of news about ethylene oxide. It seems to be a new buzzword in toxland since September 2020. But why has ethylene oxide been in the spotlight so often recently? Probably because many consumer product recalls are related to this substance. In a recent decision by the European Commission it was decided that all foods with an ethylene oxide content higher than 0.1 mg/kg should be withdrawn from the market and also withdrawn from the consumer[15]. As a result, companies were obliged to comply with this, because food safety is their responsibility.

Nevertheless, the set limit is low, and products that exceed this limit are unlikely to directly cause a public health risk[16].

Undoubtedly, a lot more toxicologically relevant news came along this summer. If you, as a NVT member, would like to share your news-related insights or involvement in the news with your colleagues, we ask you to share this with the TCDD via redactie@toxicologie.nl. ■

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16 https://nos.nl/artikel/2395326-weer-terugroepacties-vanwege-ethyleenoxide-wat-is-het-risico

Dust in IJmond contains many PAHs and metals

In Wijk aan Zee, Beverwijk, Velsen-Noord and IJmuiden, more polycyclic aromatic hydrocarbons (PAHs) and metals were found in the deposition of dust than outside the IJmond region. This has become apparent from new research by RIVM National Institute for Public Health and the Environment. The highest values were measured in Wijk aan Zee. The levels of metals, such as iron, manganese, vanadium and chromium and PAHs, were 20 to 100 times higher in Wijk aan Zee than the levels outside the IJmond region. Exposure to the quantities of lead and PAHs in the dust in IJmond is undesirable for the health of children. RIVM concluded earlier that more acute symptoms were reported to the GP, and that air quality in this region was more often poor to inadequate.

Levels of lead and PAHs in dust undesirable for children's health

Children playing outdoors and indoors come into contact with dust in the living environment. This concerns dry deposition, which can be seen on windowsills or playground equipment, for example. Children come in contact with the PAHs and metals in the dust via their skin. They also ingest it via hand-to-mouth contact. Exposure to the quantities of lead and PAHs in this dust is detrimental to children's health. RIVM National Institute for Public Health and the Environment recommends preventing exposure to these substances as much as possible. The quantities of other metals in the dust are not expected to form a health risk.

Highest levels in Wijk aan Zee

The quantities of PAHs and metals outside homes were higher in Wijk aan Zee, Beverwijk, Velsen-Noord and IJmuiden than at locations outside the IJmond region. They were highest in Wijk aan Zee. The levels of some metals, such as iron, manganese, vanadium and chromium and PAHs, were 20 to 100 times higher than the levels outside the IJmond region.

In Wijk aan Zee, the quantities of PAHs and most metals inside homes were also somewhat higher. However, these quantities were much lower than those found outdoors and contribute very little to the overall quantities to which people are exposed. Research into the origin of the substances is still ongoing. What is already clear is that some of the dust comes from the Tata Steel site.

Sweep sampling

Dust can be seen around the Tata Steel site in the IJmond region in Noord-Holland, for example, on windowsills, garden furniture and streets. This is a nuisance to local residents who are also concerned about their health and that of their children. Commissioned by the Province of North Holland, RIVM and the Municipal Public Health Service (GGD) of Kennemerland have therefore measured the quantities of PAHs and metals in the deposited dust. They have also estimated the risks for the health of children aged between one and twelve years who live and play in this area. In various villages in the vicinity of Tata Steel, dustfall samples were collected and investigated on three occasions. This was done at 29 locations outdoors and at 12 locations inside homes. In addition, research was carried out at several areas outside the IJmond region for comparison purposes. ■

Reference:

Dust in IJmond contains many PAHs and metals | RIVM

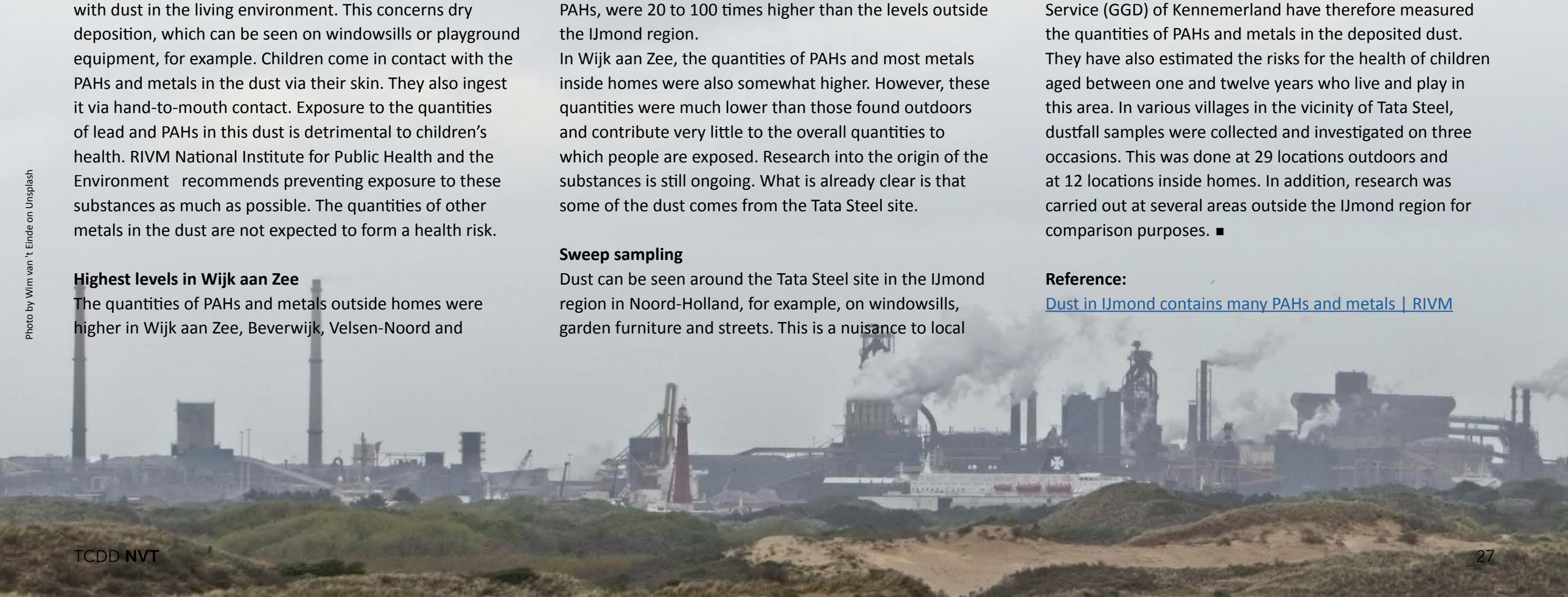

Photo by Wim van 't Einde on Unsplash

Current Concepts in Quantitative Risk Assessment for Skin Sensitization

Allergic contact dermatitis is one of the most frequent occupational diseases associated with chemical exposure. Chemicals and pesticides must be tested for their potential to cause skin sensitization and there have been major developments in testing strategies in recent years, as new approach methods are becoming accepted alternatives to traditional animal tests. Despite this progress, quantitative risk assessment of skin sensitizing chemicals remains a challenging process.

Webinar Series Co-organized by the US National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Swiss Centre for Applied Human Toxicology and the Swiss State Secretariat for Economic Affairs.

This webinar series will provide an overview of the current state of the science in this rapidly developing field. The webinars are intended as background for a planned workshop in 2022 on quantitative risk assessment of skin sensitizing pesticides but they are of interest to the broader scientific community as they are relying on the development of concepts and methods in other areas e.g. cosmetics, fragrances and consumer products.

Registration is free and open to the public.

The webinar series is organized by NICEATM, the Swiss Centre for Applied Human Toxicology, and the Swiss State Secretariat for Economic Affairs.

Register for the December 8 webinar:
"Methods for hazard and exposure assessment"

If you registered for Webinar 1, you are automatically registered for Webinar 2.

Webinar Schedule

WEBINAR 1, SEPTEMBER 21, 2021, 9:00-10:30 A.M.
EDT: INTRODUCTION TO SKIN SENSITIZATION AND CONTACT DERMATITIS.
A video from Webinar 1 will be posted here when available. All registrants will be notified by email.

Presentations:
- Skin sensitization: history and current state of play
  David Basketter, DABMEB Consultancy Ltd.
- Epidemiology of contact dermatitis with a focus on occupational exposure and monitoring of skin sensitizers
  Wolfgang Uter, University of Erlangen-Nurnberg

WEBINAR 2, DECEMBER 8, 2021:
METHODS FOR HAZARD AND EXPOSURE ASSESSMENT.

Presentations (titles subject to change)**:**
- Hazard assessment and potency determination using current non-animal test methods
- Exposure assessment and quantification

WEBINAR 3, MARCH 9, 2022:
OPPORTUNITIES AND NEW APPROACHES FOR QUANTITATIVE RISK ASSESSMENT.

Presentations (titles subject to change)**:**
- Quantitative risk assessment for skin sensitizing cosmetics and fragrances
- The road ahead: future developments and methods still in the research phase

SOT annual meeting 2022

March 27-31, San Diego, CA

Dear Colleague,

The global pandemic has informed many new ways in which we can effectively engage to share science. This is especially true for the SOT Annual Meeting. I am pleased to announce some enhancements that we are making to the 2022 SOT Annual Meeting and ToxExpo that will enable SOT to present the best research available to a wider audience.

First, the abstract submission deadline is being extended to Wednesday, December 1, to allow more time for research completion and subsequent submission (please see the note at the end of this email for information on how awards that require abstracts will be handled this year). We also have decided to make this new deadline one that we take forward for future years because giving our community more time to submit can maximize opportunities for the newest findings in our field to be considered every year.

While I look forward to seeing all of you in person March 27–March 31, 2022, in San Diego, we recognize that travel restrictions and health concerns remain for many in our community. For this reason, SOT will provide enhanced in-person meeting features that also will enable remote participation in this year's event. Although details about these additional features are still being finalized, we wanted you to let you know that our goal is to ensure that those on-site in San Diego and others spanning the globe will be able to engage with robust scientific content throughout the event.

I look forward to sharing more information with you in the coming months about continued enhancements to the in-person meeting and to engaging with you in March 2022 from San Diego.

Myrtle Davis, DVM, PhD, ATS

2021–2022 SOT President

New Abstract Deadline:
December 1, 2021

This new abstract submission deadline is an enhancement to the SOT meeting that will continue in future years.

Submit an Abstract

What about Award Deadlines?
Some awards that require an accepted abstract have application deadlines that are before the new abstract submission deadline of December 1. If you are applying for one of these awards, submit your abstract before the award application deadline. The award reviewers will obtain confirmation of your abstract submission prior to making their selection.

TERUG NAAR INHOUD

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