

SPECIAL THEME

AI and Computational Toxicology

- Exploring the potential of Artificial Intelligence (AI)
- Interview with Job Berkhout
- Computational Science in drug design and drug safety
- Toxicology entering the single cell RNA-sequencing era
- Questions asked to ChatGPT
- Potential applications of Artificial Intelligence in toxicology
- Computational Approaches Working Group (CAWG)

Colofon

Toxicologische Communicatie, Data en Documentatie

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Bestuursvergaderingen 2023

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Het lidmaatschap wordt automatisch verlengd tenzij de NVT-ledenadministratie vóór 1 december van het lopende jaar schriftelijk of per e-mail een opzegging heeft ontvangen. Hiervan ontvangt u een bevestiging.

Contributie NVT

Incl. abonnement TCDD 53,= euro

(extra kosten EEMS: 10,= euro)

Sluitingsdata kopij 2023

10 februari, 14 april,

29 september, 24 november

Kopijbus

redactie@toxicologie.nl

Website NVT

<http://www.toxicologie.nl>

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Green Bean Design, Nunspeet

www.greenbeandesign.nl



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Editorial

Our editorial team started 2023 by brainstorming for the theme for this TCDD edition. What are the hot topics in toxicology at this moment? What is currently in the news and can be easily linked to toxicology? ChatGTP was certainly a hot topic and it generated lots of attention to the current status of artificial intelligence (AI). What if we could do an update on AI in toxicology? It has been a theme issue previously (TCDD'19 ed. 3), but time flies and developments in toxicology and AI do not stand still*. So we decided to go for "AI and Computational toxicology" for the first edition of this year.

We are confident that you will enjoy this topic as we have received great theme-related content from many of you as well as from toxicologists within your network. To list a few examples: Emilio Benfenati (ONTOX) and Gerhard Ecker (RISK-HUNT3R) have summarized the key principles, successes obtained and future goals from the Computational Approaches Working Group (page 17). Benthe van de Lugt (WUR) has written an article on Exploring the potential of Artificial Intelligence (AI) for extracting toxicity data derived from New Approach Methodologies (NAMs) studies for chemical risk assessment (page 5). Also do not miss out on the interview with Job Berkhout, a PhD student at RIVM that is working on a computational model for neural tube closure defects (page 7). Making up your mind about ChatGTP might be easier after reading the articles from our editors Carolien (page 12) and Damiën (page 14, co-written with Pim

Wassenaar). And also, the Toxafette (Nina Wieland) perfectly fits the theme (page 25). The table of contents will show that there are several more very interesting theme articles, so please read them all!

To end with a personal note: this will be my last contribution to TCDD. After obtaining my PhD in toxicology and epidemiology, I started working as a toxicologist in the pharmaceutical industry. Though I'm still working in pharma, my professional career path changed course in recent years from toxicology to regulatory affairs to global clinical operations. As the editorial team benefits most from active toxicologists, it's time to say goodbye. At the same time, I am happy that I can also say Welcome! to a new member of the editorial team: Marcha Verheijen. Marcha is working as postdoc at the Department of Toxicogenomics at Maastricht University. She will introduce herself in the next TCDD. Welcome Marcha!

On behalf of the editorial board,

Maaike Steenhof

*TCDD also does not stand still. An overview of our theme editions can be found on page 28. Don't miss out on the fun facts for the latest news and try our new puzzle so you can compete for a prize!





SECTION RISK ASSESSMENT

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

In the development and production of chemicals, sustainability plays an increasingly important role. But how sustainable is the work we perform daily in our laboratories on the toxicity testing of these substances? And how can you determine and reduce the footprint of your research? The section risk assessment invites you to our annual spring symposium in which we will explore these questions with three speakers from this interesting new field.

PRELIMINARY PROGRAM

13.00 – 13.15	Welcome & Introduction
13.15 – 13.45	Speaker 1
13.45 – 14.15	Speaker 2
14.15 – 14.30	Coffee break
14.30 – 15.00	Speaker 3
15.00 – 15.30	Discussion & wrap-up



You can register for this meeting by sending an email to Kim Doornebosch (kim.doornebosch@rhdhv.com). Please report "Registration NVT spring symposium – 4 April" in the title of the email and report your name and affiliation in the email itself. After registration you will receive a link to the online meeting via MS-Teams.



SECTION OCCUPATIONAL TOXICOLOGY

Summary symposium Substance group approach for workplace hazard and risk assessment

A summary (in Dutch) was made of the symposium organized by the Section Occupational Toxicology of the NVT and the Contact Group Health and Chemistry, on the subject of: Substance group approach for workplace hazard and risk assessment. Symposium date: 10th of March 2022.

The reference of the summary: Tijdschrift voor toegepaste Arbowetenschap 2022:35(4), 184-187. The authors are Kelly Caris and Henri Heussen, Board members section occupational toxicology.

Tijdschrift voor toegepaste Arbowetenschap (TtA) can be accessed [here](#).

Exploring the potential of Artificial Intelligence (AI) for extracting toxicity data derived from New Approach Methodologies (NAMs) studies for chemical risk assessment



By *Benthe van der Lugt*
– Division of Toxicology –
Wageningen University &
Research

An important strategic goal of the European Food Safety Authority (EFSA) is to increase its use of toxicity data derived from non-animal studies, *i.e.*, New Approach Methodologies (NAMs), in its risk assessment reports. However, this poses a challenge for the risk assessor, given the vast amount of NAM-data from disparate sources. It is expected that the application of Artificial Intelligence (AI)-based strategies can aid EFSA search, review, extract and integrate NAM-based toxicity data. EFSA therefore initiated the AI4NAMs project, in which Wageningen University, the German Federal Institute for Risk Assessment (BfR) and the consultancy firm *d-fine* collaborate. The goal of the project is to investigate the potential of AI to collect relevant NAM-based toxicity data from chemical databases (*i.e.*, CompTox and PubChem) and scientific publications. Ultimately, the goal is to be able to integrate the extracted NAM data in adverse outcome pathway (AOP)-like knowledge networks, which supports the inclusion of mechanistic data into the risk assessment process.

A shisha pen contains no tobacco and usually no nicotine.

First, a set of available AI tools was reviewed based on their potential to support in a series of pre-defined tasks, including the search, review and extraction of NAM-derived data. The methodologies and results of this review were recently published in the EFSA journal [1]. The most promising tools were implemented in six case studies and their performance and usability were evaluated. Based on title and abstract, the tools DistillerSR [2] and Sysrev

[3] were tested for their ability to select eligible scientific publications derived from search engines, such as PubMed and Scopus. The models were first trained on manually selected publications, *i.e.*, expert-based screening of publications based on inclusion and exclusion criteria. After three training sessions, the tools' performance was evaluated by comparing the included and excluded publications by the AI tool and the expert. Both DistillerSR

and Sysrev were able to successfully include most publications. However, the ability of the tools to deselect non-eligible publications should be improved, given the false inclusion rates of 25% for DistillerSR and 40% for Sysrev.

Another task for which an AI-supported approach was tested was the automated extraction of NAM-derived toxicity data from scientific publications. For this task, the extraction of key elements was evaluated, such as the test substance, test system and the type and magnitude of the measured effect. Here, some of the AI tools tested, that apply a rule-based approach for the detection of key elements (such as medSpacy [4]), clearly struggled. Most likely, the large heterogeneity in the way authors report toxicological data in scientific publications contributes to the poor performance of AI tools, as it is impossible to implement a 'one-size-fits-all' rule.

Further investigation focused on the use of pre-trained language models, such as GPT-3 (Generative Pre-trained Transformer 3) [5]. Importantly, this model should not be confused with its little brother ChatGPT, which is much less powerful. GPT-3 can be applied for, amongst others,

text completion, summarisation and question answering. In order to evaluate the performance of GPT-3 in the automated extraction of key information from scientific publications, relevant questions (e.g. “*What were the test substances used in this study?*”) together with the publication text were imported in the model. The model output was found to be highly heterogenous in terms of answer format. For example, the answers varied from complete sentences or single words to answers in tabular form. Importantly, this type of output would require post-processing steps, which is time-consuming and therefore hampers the efficiency of the process. As a strategy to improve the model output, it was investigated whether fine-tuning (i.e., re-training) the GPT-3 model could improve the model output. To this end, training data was collected by manually extracting key information from 80 relevant scientific publications. The training data was provided to the model in tabular form, with each row containing linked entities (e.g., the test substance and the corresponding test system), to allow the model to be trained on this desired output format. After fine-tuning, the model successfully returned all answers in tabular format and with linked entities. This result can be considered as a major improvement of the fine-tuned model, compared to the rather unstandardized output provided by a non-fine-tuned model. Although the accuracy of the model output was highly dependent on the complexity level of the question, it can be concluded that the fine-tuning process adds to the quality of data extraction. Eventually, a more efficient handling of the extracted results is foreseen, as time-consuming (manual) post-processing actions can be highly reduced. However, it should be taken into account that manual effort is still required to obtain a fine-tuned AI model.

Altogether, the results of the AI4NAMS project provided useful insights in the potential of AI for the search, review, extraction and integration of NAM-derived toxicity data. Although there is still room for optimization of the tested AI tools, the results could serve as a starting point for further studies investigating the implementation of AI to support chemical risk assessment.

References:

- [1] Blümmel, T., et al., Review of state-of-the-art AI tools and methods for screening, extracting and evaluating NAMs literature in the context of chemical risk assessment. *EFSA Journal* 2022;20(1):EN-7815
- [2] <https://www.distillersr.com/>
- [3] <https://sysrev.com/>
- [4] <https://github.com/medspacy/medspacy>
- [5] <https://platform.openai.com/docs/models/gpt-3>



Interview with Job Berkhout

PhD Candidate at Rijksinstituut voor Volksgezondheid en Milieu

What is ONTOX and how does your project fit in?

ONTOX is part of the ASPIS cluster that represents Europe's effort towards sustainable, animal-free and reliable chemical risk assessment. ONTOX will deliver a generic strategy to create innovative new approach methodologies (NAMs) in order to predict systemic repeated dose toxicity effects that, upon combination with tailored exposure assessment, will enable human risk assessment. For proof-of-concept purposes, focus will be put on the development of ontology-based testing strategies for 6 case studies addressing adversities in the liver (steatosis and cholestasis), kidneys (tubular necrosis and crystallopathy) and developing brain (neural tube closure and cognitive function defects) induced by a variety of chemicals. My project focusses to create NAMs for the neural tube closure case study.

Why develop a computational model for neural tube closure defects?

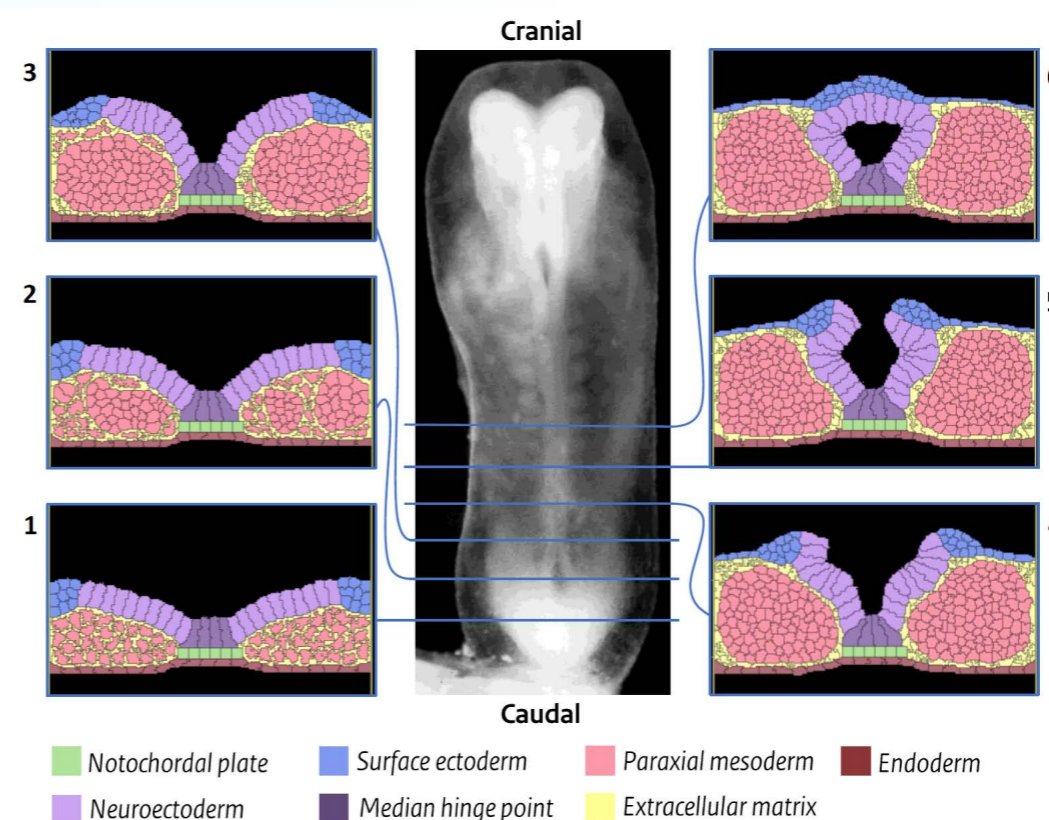
The neural tube closure is a critical process that occurs early in development, around day human gestational day 27. Neural tube closure is a complex process known to be susceptible to chemical perturbations. Failure to close results in spina bifida and other neural tube defects (NTD), which are among the most prevalent human congenital malformations. Currently human and environmental safety assessment of chemicals relies primarily on legally required *in vivo* studies in animals. Ethical concerns, the need for greater chemical coverage

whilst utilizing less resources, and growing insight in the limited predictability of animal models for human health, drive the need to find alternatives to animal experiments. By simulating a complex biological process such as neural tube closure, we aim to demonstrate that computational models of biological processes will provide a revolutionary approach to chemical safety assessment in the near future with less reliance on animal testing.

Could you provide some additional explanation about the computational model?

The computational model we are developing is a multicellular, agent-based model, based on a physiological map of human neural tube closure. This map revolves around the all-trans-retinoic acid (ATRA)-related molecular pathways for neural tube closure and disruption. Related morphogenetic events are recapitulated in the CompuCell3D.org modelling environment by translating the signalling cascades in the physiological map into a computational model that depicts the physical aspects of neurulation. The model can be used to predict the effects of simulated chemical exposure on developmental

Figure 1: The computational model of neural tube closure, built in CompuCell3D. The centrally placed overview of a developing embryo can be considered a snapshot of the progressive closing neural tube. Neural tube closure starts with a flat ectoderm in which the notochordal plate (green) will trigger formation of the median hinge point (dark purple) causing the first invagination of the neuroectoderm (light purple) (1,2). The paraxial mesoderm (light red) will start to proliferate, further increasing the invagination, and form condensed cell structures (3). Then the bending of the neuroectoderm will occur following formation of the dorsolateral hinge points (4, 5). Subsequently, the neuroectoderm and the surface ectoderm (blue) will fuse, closing the neural tube (6).



processes critical for neural tube closure, such as median- and dorsolateral hinge point formation.

Do you think that AI would one day help in chemical risk assessment? What would be the benefits and potential pitfalls?

I think that AI definitely has the potential to improve chemical risk assessment. When considering the large amount of chemicals that yet need to be properly assessed, an approach as powerful and cheap as AI will certainly benefit chemical risk assessment, with, in the least, the creation of a chemical priority list. AI approaches are rapidly developing and emerging in various areas of toxicology, and some of them already show a higher reproducibility compared to OECD approved animal tests. Especially in hazard identification where potential hazard of a chemical is predicted based on its molecular structure. Yet such predictions face difficulties as the field deals with unknowns; e.g. what is the actual response when a certain chemical binds to a certain receptor. Applicability of AI in this area is still largely under development as sufficient and reliable datasets to train models on are lacking. However, the usage of AI for chemical risk assessment lies not in toxicological predictions alone. AI can assist with making sense of the ever-growing toxicological data landscape.

Could you tell us more about the awards that you have won?

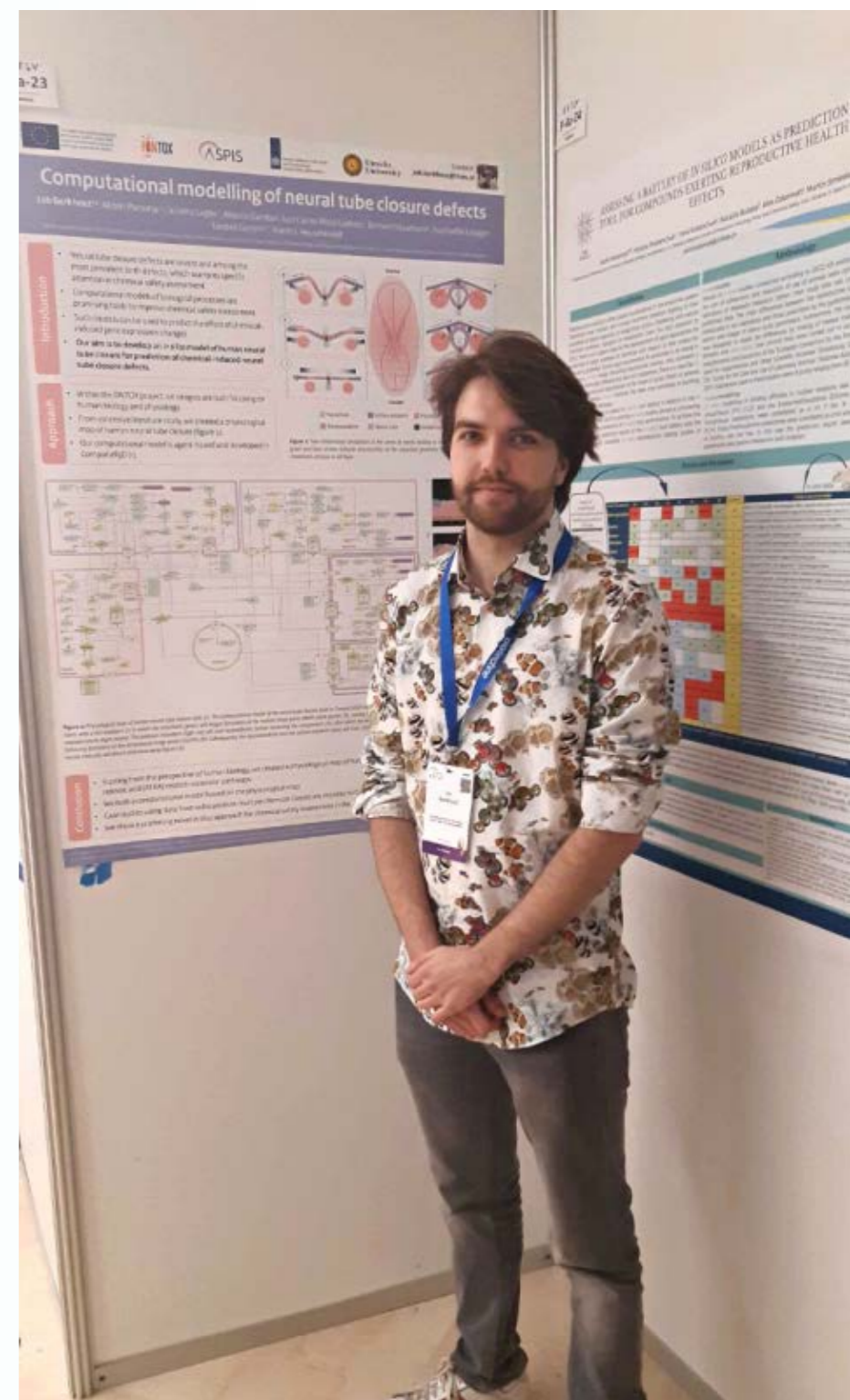
With my research project titled: Computational modelling of neural tube closure defects, I've been honoured with a EUROTOX Early Career Award at ICT 2022 (award category: ECETOX Christa Hennes Award for toxicological research into mechanisms and risk assessment), and the ESTIV 2022 Best poster award at ESTIV 2022 Congress in Barcelona, Spain.

What motivates you and what are your ambitions for the rest of your PhD project and beyond?

I really enjoy working on my project. The pioneering aspect fits me well and I have great colleagues. I appreciate and stand behind the vision of ONTOX; to advance human risk assessment of chemicals without further use of animals. What lies beyond I do not know yet. That's something I will look into once I enter the final year of my PhD.

To end on a lighter note, who are you as a person when you are not the aspiring scientist? What are your hobbies and interests?

I am generally seen as an enthusiastic and energetic person, always in for social activities. I enjoy being creative as well. I play the guitar, mainly acoustic, and enjoy writing songs myself. I also like to paint, although I am not really good at it. Besides this, I relish visiting festivals for the music and the ambience. I also like to meet with friends for drinks or game nights. We play video games or more complicated games, like Dungeons and Dragons.



Computational Science in drug design and drug safety

Scope and purpose of AI and Computational Science

Artificial Intelligence (AI) is a branch of computer science that deals with the development of intelligent machines that can perform tasks that would normally require human intelligence, such as recognizing speech, making decisions, and solving problems.

Computational models, on the other hand, are mathematical representations of systems or processes. In AI, computational models are used to represent and simulate the workings of the human mind, such as perception, reasoning, and decision-making. These models can be used to make predictions and generate outputs based on input data.

So, the difference between AI and computational models lies in their scope and purpose. AI is a broader field that aims to create intelligent machines and systems, while computational models are specific tools used within AI to represent and simulate specific aspects of intelligence.

Computational models can be used to simulate the interactions between drugs and biological targets

Computational models have been used in drug design for several decades and they play an important role in the discovery and development of new drugs. These models can be used to simulate the interactions between drugs and biological targets, such as proteins, and to predict the efficacy and toxicity of new compounds.

AI has been increasingly applied to drug design, and it has the potential to revolutionize the field. AI algorithms can be used to analyse large amounts of data and identify new drug candidates, predict their properties and behaviour, and optimize their design. This fits the scope of “safe by design” effort made by industries. For example, AI algorithms can be used to screen large libraries of compounds for potential drug candidates, predict the binding affinity of a drug to its target, and design new drugs with improved properties.⁵

Machine learning methods are a type of artificial intelligence that allow a computer to learn and make predictions based on data. Some commonly used machine learning methods in drug discovery include:

Quantitative Structure-Activity Relationship (QSAR)

methods: QSAR models are used to predict the biological activity of a chemical compound based on its molecular structure. These models are built by training a machine learning algorithm on a set of chemical compounds and their known biological activities, and then using that model to predict the activity of new compounds.⁶

Support Vector Machines (SVMs): SVMs are a type of machine learning algorithm used for classification and regression problems. In drug discovery, they are used to classify compounds as active or inactive against a particular target, or to predict the potency of a compound against a target.

By Carolien Schopuizen



Random Forests: Random Forests are a type of machine learning algorithm that is used for both classification and regression problems. They are based on the idea of decision trees, where the algorithm makes a series of binary decisions to arrive at a prediction. In drug discovery, Random Forests are used for tasks such as toxicity prediction, Absorption, Distribution, Metabolism, and Excretion (ADME) prediction, and target prediction.

These machine learning methods are used in drug discovery to help researchers identify promising drug candidates and make more informed decisions about which compounds to pursue in further studies. By analysing large amounts of data, these methods can help researchers to identify



patterns and relationships that may not be apparent in individual experiments, allowing them to make more accurate predictions about the potential activity of a compound.²

Advantages and disadvantages of applying computational models in drug development

The use of computational models can increase speed and cost-effectiveness: Computational models can analyse large amounts of data much faster and more efficiently than traditional experimental methods, potentially reducing the time and cost of drug development. Furthermore, they can reach increased accuracy. By analysing large amounts of data, computational models can identify patterns and relationships that may not be apparent from individual experiments. Thereby allowing for (more) accurate predictions about the potential activity and safety of a compound.

Also, computational models can be used to predict the toxicity of a compound before it is tested on animals, potentially reducing the number of animals used in the drug development process. And they can play a role in improved decision making. Computational models can help researchers to make more informed decisions about which compounds to pursue in further studies – thereby potentially reducing the risk of pursuing a compound that is unlikely to be effective or safe.

Additionally, these models may aid in the design new molecules that are more sustainable regarding production as well as for the environment. This fits the scope of “safe and sustainable by design” developing in industries. However, there are also some limitations to the use of computational models in drug development and safety, including:

The accuracy of a computational model is dependent on the quality and size of the training data. If the training data is of poor quality or limited in size, the model may not be accurate. Furthermore, computational models may suffer from a lack of understanding of complex biological systems: while computational models can help to identify patterns and relationships in data, they may not fully capture the complex biological systems that underlie drug development and safety.

There also is still a need for validation when applying computational models. They should be validated against experimental data to ensure their accuracy and reliability and avoid bias. Computational models can be biased if the training data contains systematic errors or if the model has been built with a particular outcome in mind. It is important to ensure that the training data is diverse and representative to avoid bias. And there is a need for regular updates: as new data becomes available, computational models may need to be updated to account for changes in the understanding of the biological processes involved in drug action and toxicity.

Overcoming a Black Box character of the model

The black box character of computational models can be challenging, as it can make it difficult to understand the basis for the predictions, and thereby hinder interpretation (for example when applying QSAR modelling in safety prediction).⁴ However, there are strategies that can be used to overcome this issue and make the models more transparent such as model interpretation methods.¹ For example: explainable AI (XAI): XAI is a new field of research that aims to develop machine learning models that are both accurate and transparent.³ XAI models use techniques such as rule-based systems, decision trees, and transparency-

enhancing regularization to make their predictions more interpretable.³

It is interesting to note that there is often a trade-off between model accuracy and interpretability, and finding the right balance can be challenging. Who knows, in some cases, it may be necessary to compromise on accuracy to make the model more transparent, while in other cases, it may be possible to maintain high accuracy while still making the model more interpretable. The best approach will depend on the specific needs of the problem being addressed.

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5. **Paul, D., Sanap, G., Shenoy, S., Kalyane, D., et al.**, Artificial intelligence in drug discovery and development. *Drug discovery today*, 2021. 26(1): p. 80.
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Toxicology entering the single cell RNA-sequencing era

The department of Toxicogenomics at Maastricht University has been focusing on high throughput RNA-sequencing technologies for over a decade. By participating in international projects, expertise has been obtained in expression analysis of messenger RNA's (both on gene and isoform level) and non-coding RNAs (e.g. microRNAs, circulating microRNAs, circular RNAs, etc.).



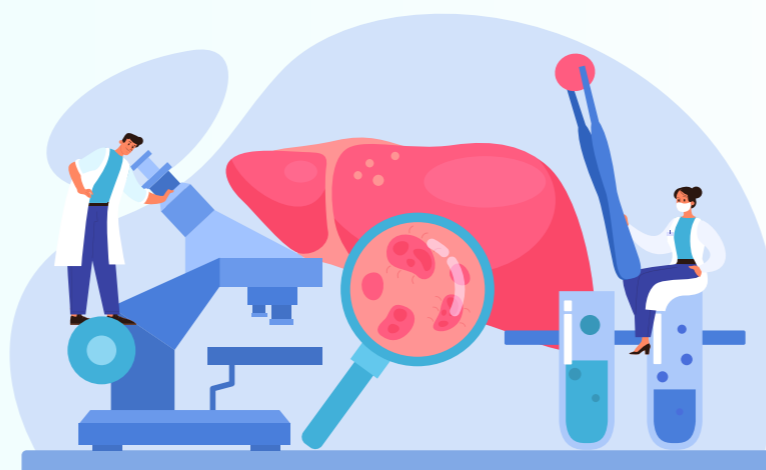
By Marcha Verheijen

Other fields of research, such as cancer research, have been advancing greatly in their technological capabilities and are now applying high throughput sequencing techniques to single cells. It would be a great opportunity for the toxicology field to use these new technologies for obtaining a more in-depth insight into molecular effects. To this end, **Dr. Florian Caiment** and **Dr. Twan van den Beucken** have generated single cell RNA sequencing data of exposed primary human liver 3D co-cultures. I consider myself very lucky to be able to work together with them to optimize single cell RNA bioinformatics pipelines to ensure their applicability in the toxicology domain. Let me tell you why.

RNA sequencing technology has the capability of measuring all expressed RNAs in a sample, making it a valuable source for in-depth analysis of gene-expression changes resulting from a toxicological exposure. It is especially suited for hypothesis generating research, thereafter, followed up by targeted validation experiments. However, most of the available toxicological transcriptomics data are gained from bulk-sequencing, meaning that the transcriptomes of many cells (either from culture or tissue samples) have been measured together. This means that the resulting gene expression reflects the sum of all cells and not the expression in individual cells. This is a major drawback, because many

samples contain multiple cell types and even if a single cell type is investigated, we all know there is major heterogeneity between cells. Single cell sequencing, as the name already implies, solves this issue by measuring transcriptomes of single cells and presents with other benefits and opportunities.

Many *in vitro* toxicological studies use a small number of replicates (often triplicates) which reduces the statistical power of the analysis. But when you measure all the individual cells in these samples (which can be hundreds or even thousands), the N increases vastly and with it the statistical power of the analysis. Next to statistical advantages,



there are many more biological research opportunities. With single cell RNA data, it is possible to group cells according to their expression profile. These groups can be different cell types (as would be the case in co-cultures or tissue samples), but you could also identify cells reacting differently to the exposure within a single cell type. Next to these applications, you could also investigate cells or cell types that interact with each other through the analysis of ligand-receptor interactions and see if an exposure can change this interaction. Furthermore, when time series are analysed, one could for instance apply a trajectory analysis to monitor processes like apoptosis, carcinogenesis, or developmental processes.

Already these additional research opportunities make me very excited to work with single cell RNA data. This is only the tip of the iceberg. Increasing the number of replicates also enables the possibility to apply machine learning methods to this data. This opens up the door to many more analysis strategies, more than I can imagine at the moment. However, before we can apply all these amazing analyses, as said before, we should first optimize single cell RNA bioinformatics pipelines to ensure their applicability in the toxicology domain. That is what the department Toxicogenomics at Maastricht University is currently working on, and we aim to have a first publication later this year.

Questions asked to ChatGPT

The future is here...or is it? Recently a chatbot has become publicly available, that produces an on-demand reply based on your preference. It answers all our questions, varying from the production of a sonnet on the distillation of alcohol, to the production of a short essays and book chapters. Please find below some questions to see how ChatGPT views itself in the field of toxicology. And judge for yourself...

What is ChatGPT?

GPT (Generative Pretrained Transformer) is a type of language model developed by OpenAI. It is a machine learning model that has been trained on a massive amount of text data, allowing it to generate human-like text and respond to natural language prompts in a conversational manner.

ChatGPT, as the name suggests, is a specific implementation of the GPT model that is designed for conversational AI applications, such as chatbots or virtual assistants. The model has been trained on a large dataset of conversations and is capable of generating natural-sounding responses to a wide range of questions and prompts.

ChatGPT can be used to build conversational AI applications that provide information, answer questions, and perform various other tasks. The model can be fine-tuned for specific use cases, such as customer service, personal shopping, and more.

Overall, ChatGPT is a powerful tool for building conversational AI applications, and it has the potential to revolutionize the way we interact with technology.

Is ChatGPT fact checked?

As a language model developed by OpenAI, ChatGPT generates its answers based on the patterns and relationships it has learned from the training data it was exposed to. The training data consists of a diverse range of text from the internet, which may include both accurate and inaccurate information.

However, OpenAI has taken steps to ensure that the training data is of high quality and that the model has a general understanding of the world. For example, the model has been trained on a diverse range of sources, including reputable news organizations and scientific journals, in order to help ensure that its answers are based on accurate and up-to-date information.

That being said, it's important to keep in mind that AI models like ChatGPT can make mistakes, and that the answers it provides should not be considered definitive. Before taking any action based on information provided by ChatGPT, it's always a good idea to verify the information with multiple reliable sources.

In conclusion, while ChatGPT's answers are generated based on the best available information, they may not always be accurate or up-to-date, and should be fact-checked before being relied upon.

We asked (in triplicate) if AI generated advice can replace expert advice regarding toxicology. To see how chatGPT judges its own capabilities.

1. Do you think AI generated advice can replace expert advice regarding toxicology?

No, AI-generated advice cannot replace expert advice regarding toxicology. While AI models can be trained on large amounts of data, they lack the depth of understanding, experience, and context that comes from years of education and hands-on work in the field of toxicology. Additionally, the complexity of the human body and the way it interacts with different toxins requires a nuanced and holistic understanding that AI models may not have.

Expert toxicologists have access to a wealth of knowledge about the toxicity of different substances, the mechanisms by which they cause harm, and the ways to diagnose and treat toxicity. They can also consider individual factors such as a person's medical history, environmental exposure, and current health status to provide tailored and accurate advice.

AI models, on the other hand, can only provide general advice based on the data they have been trained on. They can't account for the unique circumstances of each person and may not always provide the right advice.

In short, while AI models can provide a valuable source of information, they cannot replace the expertise and experience of a trained toxicologist. When it comes to matters of health and safety, it's always best to seek the advice of a qualified expert.

2. Do you think AI generated advice can replace expert advice regarding toxicology?

As an AI language model, I do not have personal opinions or beliefs, but I can provide some information based on current research and development in the field of AI and toxicology.

AI has made significant progress in many fields, including toxicology, but it is not yet at the point where it can replace expert advice in this area. AI can provide quick access to vast amounts of information, help identify patterns and relationships in data, and assist in decision-making, but it still lacks the expert knowledge and experience that human toxicologists have.

AI-generated advice can be a valuable tool to supplement human expertise, but it is important to keep in mind its limitations and to seek expert advice when necessary. It's always important to seek advice from a qualified professional in a relevant field and to consider multiple sources of information when making important decisions related to toxicology or other health-related matters.

3. Do you think AI generated advice can replace expert advice regarding toxicology?

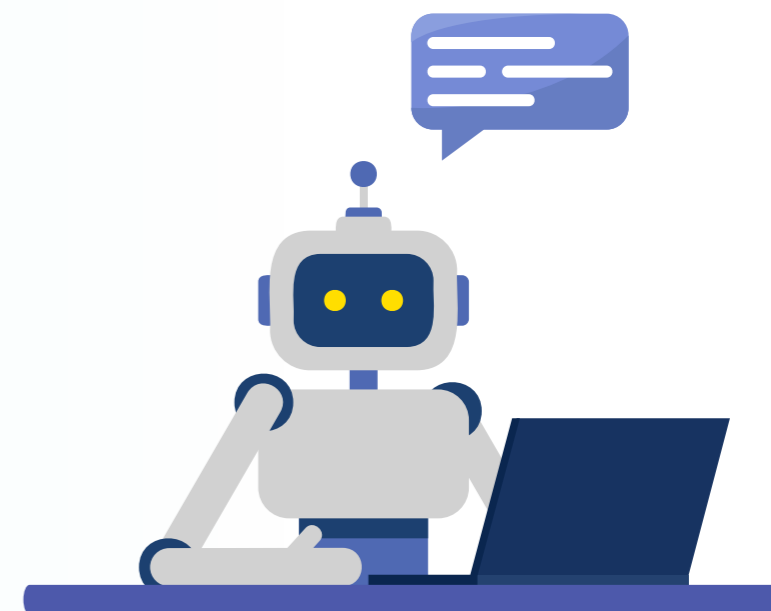
As an AI language model, I do not have personal opinions, but I can tell you that AI generated advice has the potential to complement expert advice in the field of toxicology. However, AI is not yet advanced enough to replace expert advice entirely.

Toxicology is a complex field that requires a deep understanding of both the chemistry of toxic substances and their effects on the human body. Currently, AI models have limited capability to understand the complexities of toxicology and to make informed predictions and recommendations.

Additionally, toxicology often involves making decisions based on limited information and a certain level of uncertainty, which is challenging for AI models that are based on mathematical algorithms and probability estimates.

That being said, AI can be used to assist experts in toxicology by providing quick access to large amounts of information and data. It can also be used to identify patterns and trends in data that may not be immediately obvious to humans. However, the final decision and interpretation of the information must still be made by a qualified expert.

In conclusion, AI has the potential to play a role in the field of toxicology by assisting experts, but it is not yet advanced enough to replace their expertise.



So, interestingly the Chatbot replies with 3 different answers to the same question. Though, the reasoning it provides is similar. All three times it concludes that AI has potential as an information source, can be used to assisting experts or supplement human advice. But it is not to be used as a replacement of trained toxicologists, yet...

If you are interested in reading another example of the use chatGPT, please check out the article from Damiën van Berlo and Pim Wassenaar on page 14.

If you are interested to challenge (or use) chatGPT yourself, go to: <https://chat.openai.com/chat>

Potential applications of Artificial Intelligence in toxicology

By Damiën van Berlo and Pim Wassenaar

According to the Cambridge Dictionary, intelligence is defined as “the ability to learn, understand, and make judgments or have opinions that are based on reason.” This answer immediately spawns a follow-up question: what is reason? According to this same dictionary, reason can be defined as: “the ability of a healthy mind to think and make judgments, especially based on practical facts.” Great... but then how to define a “mind”? “the part of a person that makes it possible for him or her to think, feel emotions, and understand things”. But then, what is thinking and understanding? Intelligence? Then we are back at our starting point...

What becomes clear from the previous paragraph, is that it is quite difficult to accurately define intelligence; the expectation might then be that it must be difficult to define artificial intelligence (AI) as well. The same dictionary defines that as: “*the study of how to produce machines that have some of the qualities that the human mind has, such as the ability to understand language, recognize pictures, solve problems, and learn.*” Thus, it involves the development and study of machines that can perform complex tasks normally performed by humans.

One of the most common forms of AI that has been receiving a lot of press, is machine learning (ML). It is in fact so common that it is often used interchangeably with AI. This concept is based on the ability of a machine to learn using past observations without being explicitly programmed; it has such an enormous potential to extract useful information from huge datasets, that it is difficult not to become excited by what it can do. There is nothing wrong with that excitement... as long as we are mindful of its shortcomings.

A ML approach is not inherently good or bad, it can add a lot of value and fidelity to a model but this depends on the question asked and the availability of data; whether a ML algorithm is useful, is determined by the quality and quantity of the data that are used to train the model. When very little or poor-quality data is available, a ML model would still sound like a fancy approach but would be quite useless. Such models generally follow the principles of “garbage in, garbage out” and “use only the necessary complexity, no more”; the model should be as simple as it can be to answer the question. And even a brilliant model based on lots of high-quality data cannot predict accurately what will happen when completely new input data is provided (e.g., a very new class of chemicals). It is crucial to accurately describe the boundaries of the prediction domain of a ML algorithm. And when we want to apply AI/ML in regulatory toxicology, there are certain requirements that are to be met: transparency, standardization and interpretability are important criteria that need to be addressed somehow.

When using our “natural intelligence”, we come to the following possible applications AI/ML might have in regulatory toxicology/risk assessment:

Identification of substances of concern for risk assessment:

- There are many chemicals on the market, of which many have a limited amount of toxicological data, and there are not enough risk assessors by far to evaluate hazards and risks that might be associated with all of these. Here, AI/ML can offer its artificial helping hand, e.g., by identifying and prioritizing those chemicals that might require further evaluation, by ECHA or member states;
- Quantitative Structure-Activity Relationship (QSAR) methods – which actually can be considered as a ML model – predict the activity of a substance based on its structure. The models can be elaborated by including other factors such as physicochemical properties or readouts from basic bioassays, and could be extended to multitask predictions by including more and a wider variety of data. As such, more complex patterns and relations could potentially be identified by ML approaches, thereby enhancing their predictive potential to pinpoint substances for which a concern might exist based on similar substances;

Facilitation and innovation of risk assessment practice:

- Contributing to more consistent, transparent and robust risk assessment conclusions and regulatory decisions, e.g., by considering previous evaluations and decisions;
- Regulatory toxicologists and study directors working at a contract research organization need to write a lot of standard documents, such as reports, study plans etc. Language models, like ChatGPT, can be a useful aid for such documents and provide a draft (to be checked and corrected by the human expert) based on substance information, statistically significant study results etc. This can speed up the procedure considerably;
- An important role for AI in the transition to non-animal methods, often referred to as (the rather bland) New Approach Methodologies (NAMs), is to be expected. Many regulatory questions such as “is this chemical hazardous”, “can this substance cause cancer” or “what

is the safe exposure level for this chemical” can likely not be answered using a single NAM. Rather, a battery of tests is likely to be needed, with AI assisting in selecting the combination of tests that is considered most predictive to investigate a certain adverse outcome with a substance from a certain chemical class;

- There is a large body of published toxicology data, and in a weight-of-evidence approach to assess the toxicity of a certain substance it is important to include as much information as possible. Systematic review is a method that was originally developed for clinical trials (human medicine safety studies) and is based on a systematic, objective search strategy to pick up as much relevant literature as possible based on a chain of search terms. A disadvantage of the method is that it is time-consuming and the search terms usually generate a large body of non-useful abstracts. At Utrecht University an open-source program has been developed, ASReview Lab, that

uses a ML algorithm to assist in the selection of abstracts of literature that is likely useful for a certain query. It provides abstracts to the user, who then has to indicate whether the abstract meets the inclusion criteria or not, with each subsequent decision the algorithm is optimized and suggests another abstract that most likely meets your inclusion criteria. Such approaches can be used to support extensive literature searches by saving time through ensuring that (almost) all relevant literature is considered.

Supporting generation of data to be used for risk assessment:

- AI/ML is very good at image analysis; when all observations by a set of renowned pathologists based on microscopic slides are used to train an AI algorithm, it is likely that the resulting model will be very proficient at correctly identifying pathological processes from microscopic slide images. Such approaches may provide accurate read-outs as long as the pathological process in question has been previously assessed by aforementioned renowned pathologists; a new type of pathology will not be predicted accurately. Also, quantification of, for instance, immunohistochemical staining or comet assay images is difficult to automate using conventional methods, but AI/ML approaches could quantify staining in e.g. a specific cell type or can score the tail intensity only in non-apoptotic/-necrotic cells (a well-known artefact in the comet assay);
- Some toxicity endpoints are based on the identification of patterns, such as behaviour analysis where animal movement is tracked and analysed by AI/ML. Such automated approaches could save time and could identify patterns/effects that were previously non-detectable;
- Interpretation of complex data, such as -omics data/ big data or 3D heatmaps, can be made much more



convenient by AI/ML recognizing patterns within such data that are difficult to distinguish for the human observer;

- AI can be used to link information from human biosensors (e.g., wearables to assess heart and lung function) to environmental monitoring (e.g., air quality) and current advanced approaches to characterize and map environmental factors and exposure that are part of the exposome field. This way, the effect of pollution episodes on human health can directly be translated into a warning message to the wearer to move to a less polluted location (e.g., indoor). Also, the contribution of the many different components of air pollution on human health can be investigated;

Probably, many more possible applications exist. As an interesting experiment, let us try and ask ChatGPT to give a similar overview; for a proper experimental set-up, we only involved “the machine” *after* writing down the above possibilities we could think of ourselves. This is the response (in *Italic*) “the machine” gave us when asked:

“Hey ChatGPT, what are the possible application of artificial intelligence in regulatory toxicology?”

Artificial intelligence (AI) has the potential to significantly impact many areas of regulatory toxicology, including hazard identification, risk assessment, and regulatory decision-making. Here are some possible applications of AI in regulatory toxicology:

1. **Predictive toxicology:** AI models can be trained on large datasets of toxicological data to predict the toxicity of chemicals that have not been tested. These models can help identify potential hazards and prioritize chemicals for further testing.
2. **High-throughput screening:** AI can be used to analyse large datasets generated from high-throughput screening assays to identify potential toxicants. This can help reduce the number of chemicals that need to be tested in more complex and expensive animal studies.
3. **Mode of action analysis:** AI can be used to identify the molecular targets and pathways that lead to toxicity. This can help improve our understanding of the underlying mechanisms of toxicity and enable the development of more targeted and effective interventions.
4. **Risk assessment:** AI can be used to integrate data from multiple sources, including toxicological, exposure, and epidemiological data, to estimate the risk of chemicals to human health and the environment.
5. **Regulatory decision-making:** AI can be used to support regulatory decision-making by providing evidence-based assessments of the potential risks and benefits of chemicals. This can help improve the speed and accuracy of regulatory decision-making while reducing the use of animal testing.

Overall, AI has the potential to revolutionize regulatory toxicology by improving our ability to identify and assess chemical hazards, reduce the need for animal testing, and make more informed regulatory decisions. However, it is important to ensure that these technologies are developed and used responsibly, with a focus on protecting human health and the environment.

Not bad at all, ChatGPT! Of course, it might have summarized possibilities that were mentioned in scientific journals, acting as a Google-on-steroids. But still, no major errors were made, although the last sentence seems vague. We will leave it up to the reader to decide which answer to the same question is better.

Of course, the possibilities mentioned by biological or machine in this (incomplete) piece are just examples, of which some might be a dot on a distant future horizon and others are more representative of our current reality. For many of these applications, a human supervisor will be necessary for quite some time to prevent problematic AI pitfalls. It will take time for humans to completely trust a computer algorithm, which can seem like a ‘black box’ to the observer. Progress is being made in ‘opening’ this black box and charting its content, with a specific focus on aspects that are essential to build trust in the use of these methodologies for regulatory toxicology, including interpretability/transparency.

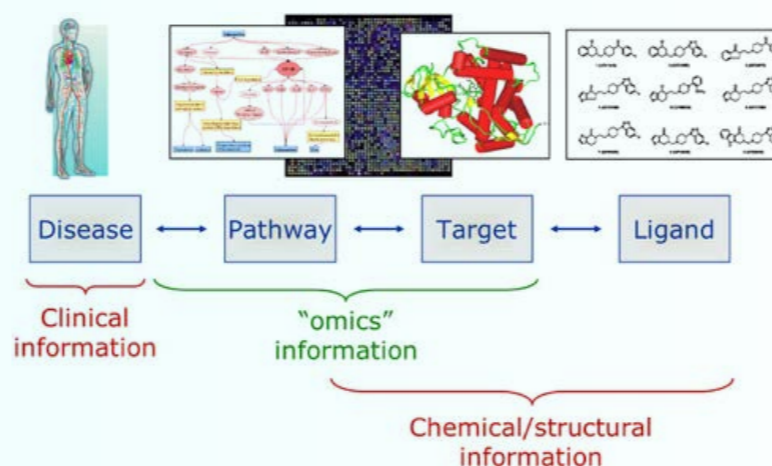
But actually, in regulatory toxicology, most decisions are based on animal tests where adverse effects are caused by exposure to a test chemical, in the absence of mechanistic corroborating information. If we trust one black box, why not another?

Computational Approaches Working Group (CAWG)

Background

ASPIS cluster is a joint collaboration of the H2020 funded projects ONTOX, PrecisionTox, RISK-HUNT3R and represents Europe's €60M effort towards the sustainable, animal-free and reliable chemical risk assessment of tomorrow. Within ASPIS, three EC funded projects cope with the development of New Approach Methodologies (NAMs). In silico models represent a key component addressed within these projects. In order to identify synergies and exploit the results in a more efficient way, ASPIS established a working group (WG) on the Computational Approaches (CAWG). These activities are focused in the following areas: (a) development of specific *in silico* models and read-across tools to evaluate properties of interest, (b) methodological studies to improve *in silico* techniques that can be applied within ASPIS - a certain method may ideally be exported from one project to another; and (c) support the activities within other WGs. Regarding the last item, the CAWG is interacting with other WGs such as the WG on Adverse Outcome Pathways (AOPs). AOPs represent a fundamental starting point in many explorations of the computational approaches, providing valuable theoretical basis. Another very important WG where interactions are planned with is the WG on toxicokinetic models, as models where computational approaches are needed are – beyond toxicodynamics - also related to toxicokinetics. The toxicokinetic aspects are very important for the quantitation of the overall effects and are therefore essential for risk assessment. The respective working

group on risk assessment is thus also a major collaboration partner for the CAWG. Furthermore, our WG will provide inputs useful for the identification of selected chemicals. A common repository of tools for specific endpoints will be created to identify the common background and the future perspectives and needs. Similarly, data associated with the models will be organised. Synergies inside the CAWG and the other WGs will be defined to increase efficiency and to maximize results.



The CAWG Modelling Approach

Co-Chairs: Emilio Benfenati (ONTOX)
and Gerhard Ecker (RISK-HUNT3R)

The key activities within the WG refer to multiple approaches with the umbrella of targeting adverse effects. These approaches range from:

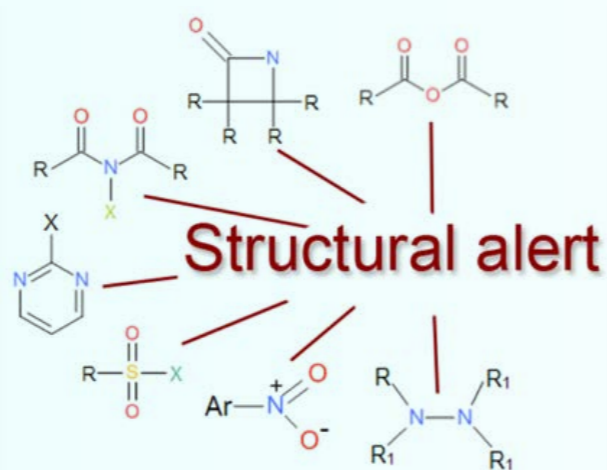
- Machine learning / Artificial Intelligence (AI). Here sophisticated methods are applied, using complex mathematical algorithms and chemical and biological descriptors in order to generate new knowledge and predictive *in silico* models.
- Data integration and -analysis to create compound/target and compound/pathway fingerprints as input for above mentioned machine learning and AI models.
- Docking methods where the 3D structure of the target biochemical enzyme is known. This allows to generate hypotheses for the molecular basis of compound binding.
- Expert systems where the information regarding pathways or molecular mechanisms are codified into rules.
- Read-across tools, where the key role is on the similarity between the target compounds and related ones, used as source.

Successes and Opportunities

As a first case study, the CAWG focused on steatosis. A first deliverable on the physico-chemical properties related to steatosis has been prepared within ONTOX, also addressing which existing *in silico* models can be used to provide suitable data. To that end, a core group of members have been identified that are actively participating in the case study. They have compiled relevant datasets and assigned specific computational tasks. The combination of different expertise within this WG provides the opportunity for consensus modelling, pooling of datasets to broaden the chemical space, and interpretations of models. The CAWG is utilising the ASPIS GitHub and interacting with the database WG (DbWG).

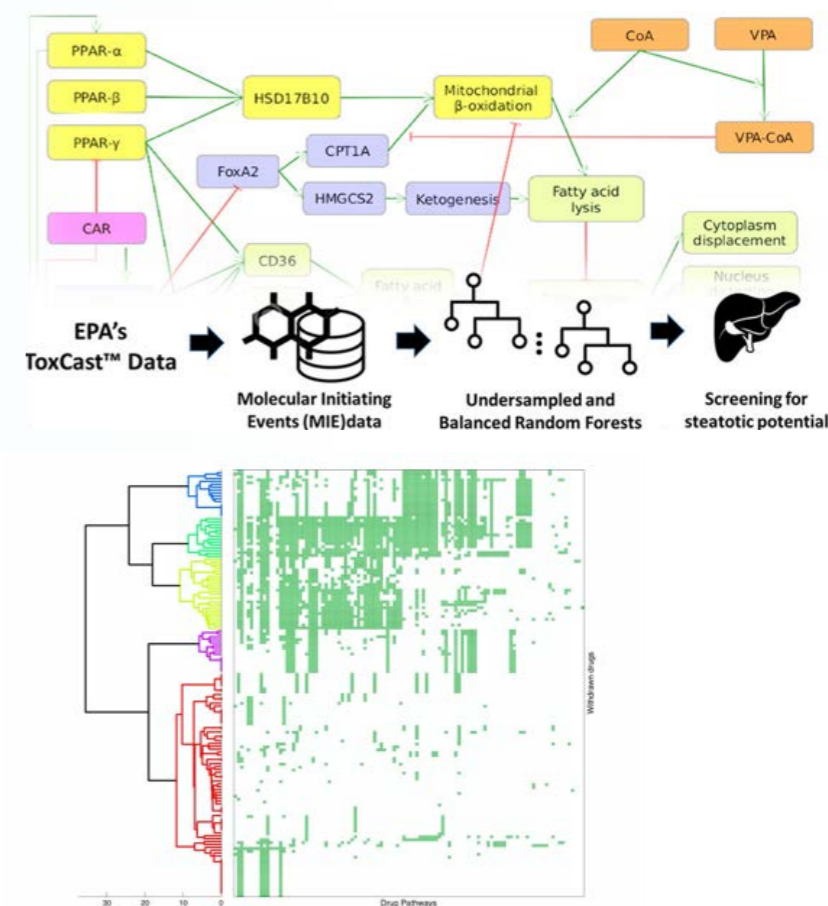
Aims for 2023

The CAWG will establish a panel of *in silico* tools for steatosis, and will address developmental neurotoxicity as a next use case. The CAWG will liaise with the risk assessment WG (RAWG) to contribute to the ASPIS Safety Profiling Algorithm (ASPA) for next generation risk assessment (NGRA).



Integrated Steatosis Model

STEATOSIS	PXR	PPAR	AKT2
Compound 1	Red	Grey	Red
Compound 2	Green	Red	Grey
Compound 3	Green	Green	Red
Compound ...			



Verantwoord omgaan met chemische stoffen: een mondiaal perspectief

Chemische stoffen zijn in beweging, zowel door de handel erin als door bepaalde eigenschappen, zoals de persistentie van sommige stoffen. Om die redenen werken landen wereldwijd samen aan het verantwoord omgaan met chemische stoffen. Voorbeelden van zulke mondiale samenwerking zijn de Verdragen van Rotterdam en Stockholm.

Het Verdrag van Rotterdam reguleert de internationale handel van elders verboden of streng in gebruik beperkte stoffen via de *'prior informed consent'* (PIC) procedure. De PIC-procedure houdt in dat de uitvoer van deze stoffen pas mag plaatsvinden na toestemming van het importerende land (formeel: verdragspartij) op basis van meegestuurde informatie over de risico's ervan. De ontvanger, vaak een ontwikkelingsland, kan zo een afweging maken tussen de risico's en het nut van een stof.

Het wetenschappelijke Chemical Review Committee (CRC) beoordeelt of zogenoemde *'notifications'* aan de vereiste criteria voldoen. Zo'n notificatie bevat de uitleg waarom een land een stof verboden of streng beperkt heeft.

In algemene zin geldt dat wanneer er tenminste twee goedgekeurde notificaties uit verschillende mondiale regio's zijn, de stof kandidaat is voor opname in het Rotterdam verdrag (*'listing'*). De bijeenkomst van verdragspartijen, de Conference of Parties (COP), besluit vervolgens over de uiteindelijke listing van een voorgedragen stof.

Het Verdrag van Stockholm heeft als doel de bescherming van mens en milieu door het beperken van productie en gebruik van persistente organische verontreinigende stoffen (POP's). Het POP Review Committee (POPRC) speelt hier

een belangrijke wetenschappelijke rol bij het voordragen van stoffen waar de COP vervolgens over besluit. Anders dan bij het Verdrag van Rotterdam streeft Stockholm naar een (uiteindelijk) mondiaal verbod van de stof.

De doelen van beide internationale verdragen zijn helder en nobel. Er zijn talrijke successen geboekt, zoals het uitfaseren van endosulfan, maar de uitvoeringspraktijk kan weerbarstig zijn. Dit laatste geldt zeker bij het Verdrag van Rotterdam waar de besluitvoering over listing in beginsel via consensus gebeurt. En wie denkt dat de meeste van de in de EU verboden stoffen ook elders uit de gratie zijn, die komt bedrogen uit.

We geven twee voorbeelden van de 'mondiale perspectieven'.

The most hated bird in Africa

In Zuidelijk Afrika vliegen er naar schatting een paar miljard (!) quelea vogels rond. Deze quelea kan zich in grote zwermen massaal op belangrijke voedselgewassen storten in Afrikaanse landen. Het dier heeft dan ook de alleszeggende bijnaam *"the most hated bird of Africa"*. Diverse Afrikaanse landen gebruiken het bestrijdingsmiddel fenthion, een avicide, om de vogel te bestrijden. De hoge

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**voormalig lid Chemical Review Committee (Verdrag van Rotterdam) en lid van de DDT Expert Group (Verdrag van Stockholm)

acute toxiciteit van fenthion maakt de stof uiterst effectief, maar veroorzaakt ook grote risico's voor mens en milieu bij gebruik. Tsjaad diende daarom een notificatie in voor opname van fenthion (strikt genomen: *'fenthion ultra low volume formulations at or above 640 g active ingredient/L'*) in het Verdrag van Rotterdam. Deze stof is in de EU ook al bijna twintig jaar verboden.



Red-billed Quelea flock at waterhole. By Alastair Rae - [1], CC BY-SA 2.0

Het CRC keurde fenthion vervolgens goed als kandidaat-stof voor listing binnen het verdrag. Echter, bij de COP stopt het proces. Landen als Kenia, Ethiopië en Soedan stellen daar dat fenthion het enige effectieve middel is om hun oogst te beschermen tegen de quelea. Hun voedselproductie kan geen verdere tegenslagen gebruiken. Zij weten dat listing geen mondiaal verbod op fenthion betekent. Maar de kans is zeer groot, zo stellen zij, dat de huidige producent stopt met het maken ervan wanneer fenthion formeel in het verdrag komt. Er is dus geen consensus binnen de COP: de stof wordt (voorlopig) niet opgenomen in het Verdrag van Rotterdam. Deze impasse duurt nu al jaren voort, ondanks VN Voedsel- en Landbouworganisatie (FAO)-ondersteuning bij het zoeken naar alternatieven voor het gebruik van fenthion.

DDT

Sinds het boek Silent Spring van Rachel Carson (1962) is er aandacht voor de negatieve effecten van DDT. Deze stof hoorde dan ook bij de eerste groep van stoffen die zijn opgenomen zijn in het Verdrag van Stockholm: de vermaarde "dirty dozen".

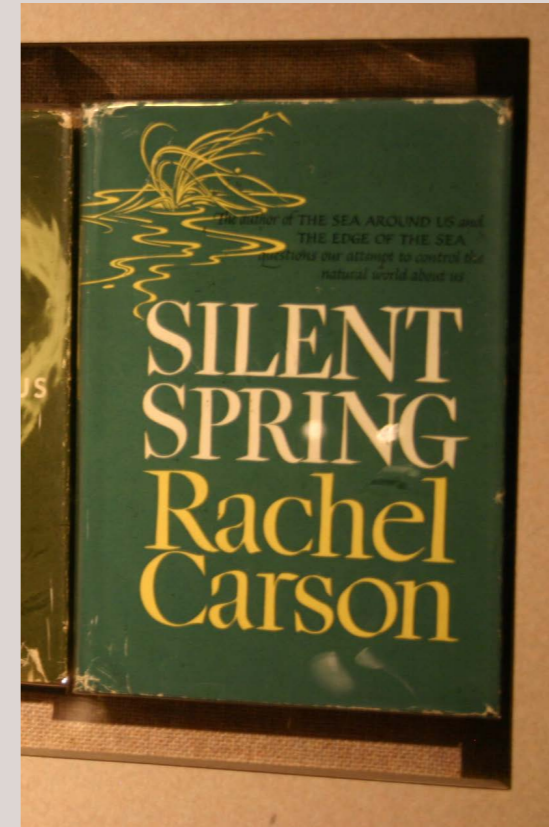
Voor DDT is er geen sprake van een algeheel verbod binnen het verdrag, maar van een restrictie. De stof mag alleen nog gebruikt worden voor het bestrijden van malariamuggen in situaties waar geen bruikbare alternatieven aanwezig zijn. Landen die de stof gebruiken dienen dit bovendien te melden in het DDT-register. Verder brengt een expertgroep elke twee jaar advies uit aan de COP over de 'voortgang' rond productie en gebruik van DDT. De groep volgt ook nauwlettend de wereldwijde situatie van malariagevallen en de beschikbaarheid van alternatieven.

Deze expertgroep stelde in november 2022 vast dat het aantal malariagevallen en -doden de voorgaande twee jaar wereldwijd toegenomen is. Dit onder meer door de gevolgen van klimaatverandering en de COVID-19 pandemie. Er zijn nieuwe alternatieve stoffen en bestrijdingsmethoden beschikbaar gekomen. De prijs van deze alternatieven is echter vooralsnog zoveel hoger dan van DDT dat ze met name voor de landen in Zuidelijk Afrika nog geen soelaas bieden. Daarnaast spelen er problemen rond resistentievorming tegen hoopvolle alternatieve stoffen en zijn er leveringsproblemen. Tot slot verloopt de sociale acceptatie van een aantal alternatieven soms moeizaam.

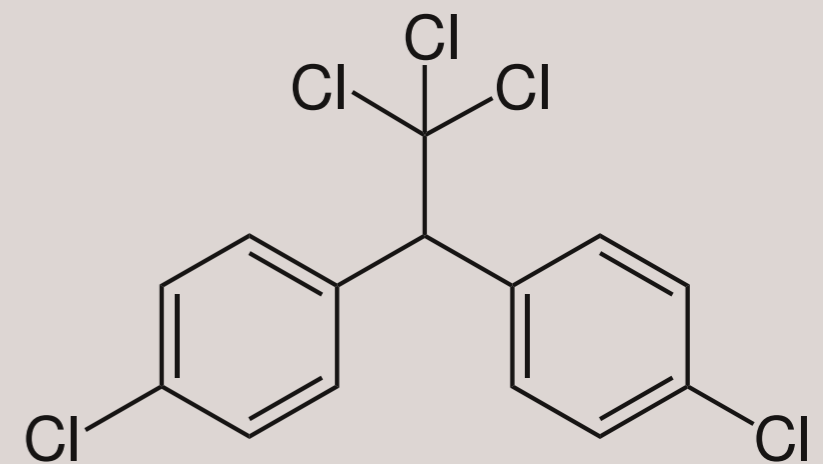
Wereldwijd uitfasen van DDT zit er op de korte termijn dus nog niet in. Er blijft werk aan de winkel voor het beschikbaar maken van betere alternatieven.

Meer informatie:

- Verdrag van Rotterdam: www.pic.int
- Verdrag van Stockholm: www.pops.int
- Endosulfan: <http://www.brsmeas.org/Portals/4/download.aspx?d=UNEP-FAO-CHW-RC-POPS-PUB-SuccessStory-2022-11.English.pdf>



"Rachel Carson: Silent Spring" by Ryan Somma is licensed under CC BY-SA 2.0.



Chemical Structure of DDT.
Leyo, Public domain, via Wikimedia Commons

Mermaid's tears flow on beaches in England and France

Mermaid's tears, a beautiful mythological name which refers to pollution by industrial plastic granules (IPGs). IPGs are used by the industry to create plastic products, they are mostly made of polymers ($\approx 90\%$) and some chemical additives ($\approx 10\%$; more than 10,000 different ones)¹. Over 80% of the polymers used are:

- low density polyethylene (PE-LD),
- high density polyethylene (PE-HD),
- polypropylene (PP),
- polyethylene terephthalate (PET),
- polystyrene (PS) and its expanded version (EPS),
- and polyvinyl chloride (PVC).



The mermaid's tears found in France shows that it is 90% polyethylene and that there are no differences observed between the different samples¹.

These so-called mermaid's tears are often lost during handling incidents along the production chain and during use (losses estimated by Eunomia range between 16,888 and 167,431 tons for the year 2018 in Europe). These operational losses in the environment cause an ambient contamination of aquatic ecosystems, IPGs are ubiquitous in regional coasts and some rivers².

To the top of that, there are also accidental losses leading to a one-off but potentially significant amounts (e.g. road or rail accidents, loss of containers at sea, container ship accidents). For example, the loss of 11,000 tons of IPGs in Sri Lankan coastal waters following the sinking of the MV X-Press Pearl in 2021. The flow on England's and France's beaches is also expected to come from a lost container at sea³.

IPGs are considered safe therefore the law does not stipulate the need to declare their transport. Additionally, there are no strict rules relating to their packaging and labelling. If a container is lost, a declaration must be made to the authorities, but no penalties are planned if this is not performed.

The properties of the IPGs make them persistent pollutants: solid, persistent, non-soluble, non-emulsifiable, non-dispersible products, non-evaporating, non-biodegradable



By Héloïse Proquin

non-adherent and mostly floating¹. Due to their buoyancy, IPGs can be disseminated in aquatic environments at a large spatial scale, potentially transboundary. On the water, the drift of floating IPGs is mainly influenced by the intensity of the winds and currents. On soils and beaches, winds, tides and runoff govern the dissemination of IPGs and their transport to waterways (amplifying the dissemination of GPIs) or areas vegetated (trapping IPGs). Wind, tide and trampling of the spill area can cause GPIs to be buried in loose soil/sediment over several tens of centimetres.

Three types of environmental impacts are suspected:

1. physical impacts on habitats;
2. ecotoxicological impacts for ecosystems;
3. risk of colonization and transport of species (e.g. colonization of GPIs by species invasive and pathogenic).

Studies are showing that some organisms have absorbed IPGs⁴ and translocated them from the gastro-intestinal track to the rest of the body^{5,6}. Other studies show that it can be transferred to other species⁷. Long term effects are lacking data, but a 2-generation study⁸ on copepods showed that microbeads of $0.5\mu\text{m}$ of polystyrene caused the death of nauplii and copepodites of the first generation at a concentration of $12.5\mu\text{g/ml}$ and of the second generation

at a concentration of 1.25 µg/ml. In the same study, developmental delay was measured at 25 µg/mL for 0.5 µm microbeads.

These types of one-time pollution are regular, the International Maritime Organization has been under pressure over this matter and is being urged to improve the rules so that these mermaid's tears belong only to mythology.

What nurdles can look like in the environment and where they can end up.
BY hockadilly, CC BY-SA 2.0, via Wikimedia Commons



References:

¹ <https://wwz.cedre.fr/content/download/10963/file/Infos-cles-GPI.pdf>

² <https://www.ecologie.gouv.fr/lutter-contre-granules-plastiques-industriels-dans-nature>

³ <https://www.euronews.com/green/2023/01/24/france-to-take-legal-action-over-plastic-pellets-causing-an-environmental-nightmare-on-its>

⁴ Imhof, H. K., Ivleva, N. P., Schmid, J., Niessner, R., Laforsch, C. (2013). Contamination of beach sediments of a subalpine lake with microplastic particles. *Current biology*, 23(19), R867-R868.

⁵ Rosenkranz, P., Chaudhry, Q., Stone, V., Fernandes, T. F. (2009). A comparison of nanoparticle and fine particle uptake by *Daphnia magna*. *Environmental Toxicology and Chemistry*, 28(10), 2142-2149.

⁶ von Moos, N., Burkhardt-Holm, P., Koihler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environmental science & technology*, 46(20), 11327-11335.

⁷ Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. *Environmental pollution*, 185, 77-83.

⁸ Lee, K. W., Shim, W. J., Kwon, O. Y., Kang, J. H. (2013). Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. *Environmental science & technology*, 47(19), 11278-11283.



Plastic pellets, industrials granules, nurdles or mermaids' tears of colors white, amber, black, red ... southwest of France (sandbeach) Plage de la côte Aquitaine (réserve naturelle nationale)
BY maldeseine, CC BY-SA 3.0, via Wikimedia Commons

The way forward in multi-omics data analyses

From the methylome to the transcriptome and the proteome in drug-induced cardiotoxicity and hepatotoxicity

Drug side effects play an important role in drug safety. Research on drug adverse effects helps to unveil cellular disturbances under treatment as well as support drug discovery and development. High throughput omics technologies have been well established and considered as affordable approaches in biomedical research, especially for drug side effect investigations. These modern technologies can systematically capture the cells' epigenetic, transcriptomic, proteomic, and metabolomics alterations. They are powerful approaches to recognize new drug-related targets and to discover drugs' toxic mechanisms on the molecular levels.

In my thesis, I explored and demonstrated different analysis approaches on 3 omics data types (MeDIP-seq, RNA-seq, proteomics) derived from human microtissues exposed to 4 different drugs. These 4 drugs are the 3 analogues from anthracyclines family, which are doxorubicin, epirubicin, and idarubicin, and 1 antibiotic drug, namely rifampicin. Anthracyclines are essential chemotherapeutic agents in various cancer types, but their adverse effects can lead to heart failure in cancer survivors. Rifampicin is an important antibiotic for tuberculosis but it can cause liver injury. Despite their adverse effects, these drugs are still popular today. Although researchers had tried to draft the toxic mechanisms of these drugs, their paradigms remain incomplete. The main theme of my thesis is to understand the toxic mechanism of these 4 drugs; besides, this thesis also aims to explore and tackle the omics analysis obstacle. Exploring the drug toxic mechanism could support the clinical usage of these drugs as well as aid the drug

discovery process, while the established omics analysis approaches could be beneficial for other researchers to improvise and analyse their own omics data analysis.

The **chapter 1** of my thesis provides a brief of omics technologies currently available for drug side effect research and discuss different analysis approaches for drugs-induced molecular alterations inside the cell. Although omics technologies have been acknowledged for their extensive data generation, it is a genuine challenge to distil meaningful information from the massive omics dataset. This chapter also discussed the existing difficulties in omics data analysis that different omics data types require particular data processing, filtering, and analysis which are not standardized yet. These aspects are the hurdles of omics data analyses and interpretation in drug side effects research.

By Nhan Nguyen



Chapter 2 of my thesis explored the epigenetic modifications related to drug-induced side effects, in this case, epirubicin-induced cardiotoxicity. I first established a workflow to analyse the methylation data (MeDIP-seq) in chapter 2.1; then in chapter 2.2, I applied this workflow to the MeDIP-seq data deriving from cardiac microtissues exposed to epirubicin. Furthermore, in **chapter 3**, I investigated how anthracycline family can impact the transcriptome and proteome and lead to adverse side effects. Chapter 3.1 analysed the transcriptome of cardiac microtissues exposed to the 3 given anthracycline analogs and specifically focused on long non-coding RNA (lncRNA) - a newly emerged biomarker type that appeared in recent research. Chapter 3.2 analyzed the proteome of the same microtissues exposed to anthracyclines. Especially, I not only detected some candidate lncRNAs and proteins as potential targets for further anthracycline-induced side effect investigation in the *in vitro* experiment but also checked these genes and protein expression in biopsies samples obtained from anthracycline-treated patients and control subjects.

While chapter 2 and 3 focused on analysing individual omics data types from epigenomics, transcriptomics to proteomics, the scope of my thesis is not limited to single omics layers, but also attempt to pursue cross-omics

interpretation. In chapter 2, I demonstrated some inter-relations in which the gene expression could be influenced by DNA methylation alterations. Chapter 3-1 addressed the importance of both coding and non-coding genes on the transcriptional level. It offers a critical perspective on the limitation of only studying the proteome or the expression of protein-coding genes at the transcriptome.

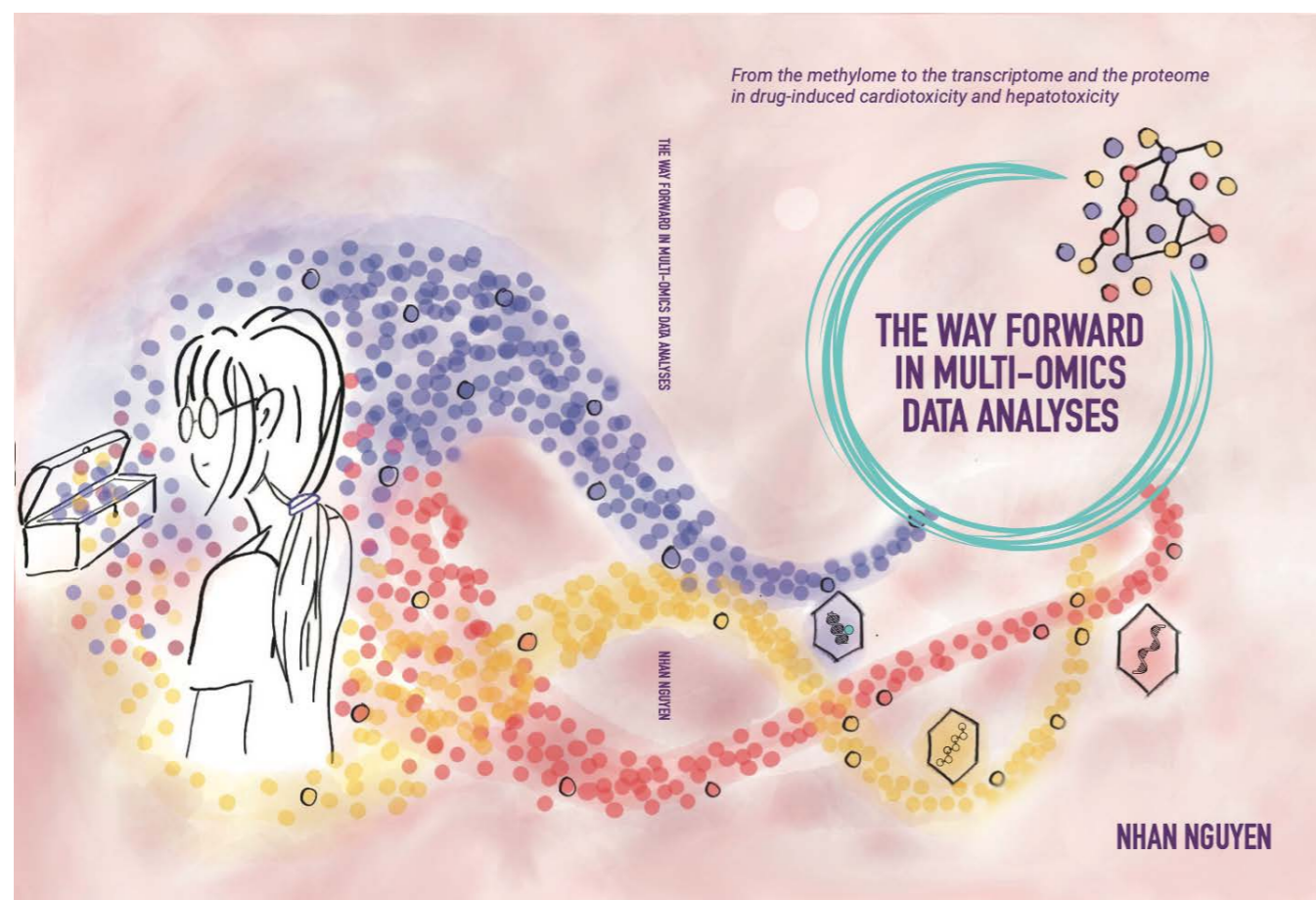
Chapter 4 is one step further in biological interaction and cross-omics analysis by focusing on transcription factors – target relationship. In chapter 4.1, I developed Regomics, a tool for downstream omics data analysis, to retrieve potentially transcription factors that regulated the given

genes/proteins. Chapter 4.2 analysed the epigenomics, transcriptomics, and proteomics from hepatic micro tissues exposed to rifampicin, a drug with hepatotoxicity side effects. The I deployed the Regomics tool to determine transcription factors – target relations possibly related to rifampicin side effects.

The last part of my thesis - **chapter 5** and the **Impact paragraph**, is a general discussion in which I compiled and recapped remarks on all of the preceding analysis research with regarding to scientific community, pharmaceutical industry and regulator's interests. The thesis suggested several potential targets and phenomena such as altered

DNA methylated genes, altered genes, lncRNAs, and proteins' expression under drug treatments. All these outcomes can be immediately used by risk assessors to evaluate the anthracycline-induced cardiotoxicity and rifampicin-induced hepatotoxicity. These potential targets can be candidate biomarkers to diagnose the particular drug's side effects and help to understand the drug's toxic mechanism. Especially, while many studies mainly focused on the proteome and protein-coding genes, this thesis emphasized the other aspects of the biological system such as DNA methylation and lncRNAs. Epigenetic modifications and lncRNAs have been implicated in cellular processes, and can also be useful tools to reveal drug-induced adverse side effects.

Furthermore, this thesis portrayed different omics analysis approaches and research angles to interpret omics data and enrich the omics analysis toolbox. The rational recognition of omics technologies requires effective and transparent data analysis approaches. This urgent need has appeared not only in the academic research community but also among regulatory agencies and in the pharmaceutical industry. Thus, all analysis approaches and source code in this thesis are publicly available (<https://github.com/NhanNguyen000>) and can already be used by other researchers. This also supports the appreciation of omics data and promotes the transparency of omics analysis. Omics analysis approaches could take advance of the modern omics technologies to demonstrate the molecular toxic mechanisms and head toward the advanced toxicology field.



DOI: doi.org/10.26481/dis.20221122tn

AIO toxafette - Nina Wieland

In the toxafette, PhD-students working in the toxicology field get the chance to share their experiences in performing research. In every issue a new candidate answers a series of questions, and then hands the baton to a fellow PhD-student.

This time the research topic fits this special issue of the TCDD. Nina Wieland, from the Radboud University has recently started her PhD research, she is studying the toxicokinetics of pesticides in humans and their translation into computational models.

Can you introduce yourself?

Hi, my name is Nina Wieland and I currently live in Nijmegen. After finishing my bachelor's degree Biomedical Sciences (BMS) in Maastricht I moved here to do my master's at the Radboud University. Alongside my studies I have always liked to be involved in extracurricular activities; from filing and organizing exams at the Faculty of Arts to being a member of the educational committee BMS. After graduating, I took a short break from studying and spent some time travelling through South-East Asia. I started my PhD at the department of Environmental Science at the Radboud University in December 2022, where I work on the EU-project Sustainable Plant Protection Transition (SPRINT) with Paul Scheepers.

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

Many people come in contact with pesticides, either at home or during their work. Farmers spray pesticides on their crops, but during this process some of the pesticide can be ingested, inhaled, or end up on the skin. Similarly, farmers' neighbours are also exposed to pesticides. Even people who don't work in agriculture or live rurally can be exposed to these substances, for example while using anti-lice shampoo, placing anti-flea bands on their pets or through eating food which has been sprayed with plant-protection products.

Even though people are often exposed to pesticides, it is unclear how the human toxicokinetics work. Current knowledge is based mostly on evidence from animal studies, but knowledge on human absorption, distribution, metabolism and excretion of pesticides is lacking.

During my PhD I will perform a human volunteer study with pesticides that are often found in human blood, urine or faeces. In this study I will expose healthy volunteers to a small amount of pesticide, after which I check how much of compound or its breakdown products end up in their blood, urine or faeces. I will use this information to create a computer model of a human, which can be used to calculate how much of the original plant-protection product has been ingested.

How is your research related to the field of toxicology, and why did you choose this subject?

My research touches upon human biomonitoring, toxicokinetics and risk assessment. I especially liked this



topic because I get to combine clinical research with computer modelling, and work with a lot of different research groups spread over Europe. There are also plenty of opportunities for me to learn new things, because my experience with toxicology prior to this PhD was focused mainly on pharmaceuticals. Pesticides are a completely new topic for me, which I think is exciting.

What was your motivation for starting a Ph.D. program?

During my internships I was supervised by PhD students all the time. The freedom that they experienced in their work, combined with the involvement in teaching and the guidance of students really appealed to me. But most importantly I didn't feel like I was done learning new things after my masters', and wanted to develop my research skills even more.

How do you see the future of your research topic (follow-up research / social impact)? What do you hope for?

I hope my computer model(s) can be implemented on a governmental level or during policy making. Most of all I hope that it will contribute to safe and sustainable food and agriculture in Europe and other parts of the world.

Would you consider qualifying yourself as European Registered Toxicologist (ERT), why or why not?

Yes, I am planning to do so. I have already started with this during my masters', where I had the opportunity to follow some of the courses required for the PET. I am sure I want to keep working in toxicology in the future, whether that is within a company or in an academic setting, so the PET will be a good start for that.

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

I am only just starting out, but compared to doing a research internship a PhD definitely gives you more responsibilities. The flexibility of being able to plan your own tasks and decide what you want to do is really nice, but can also be tricky. I'm still figuring out how to prioritize my tasks in the right way.

If you could start over your studies, what would you do differently?

I don't think I would do anything differently; I enjoyed my studies and used my internships to learn a lot of different things and figure out what I really enjoyed doing. Some students dedicate all their internships to working in the lab, but I explored multiple areas and figured out my interests in that way. I don't think one is better than the other, but I personally benefited from having a broad experience across multiple fields.

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

Guard your time and don't say yes to everything. In the beginning, everything is new and exciting. You can feel inclined to take up a lot of side-projects next to your PhD, which can be very fun but also eat away your time. It's fine to do other things alongside your PhD, but keep in mind to leave time for your primary research tasks.

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

I haven't given this much thought yet, but I would definitely like to work in toxicology on a national level or with a pharmaceutical company, focusing on toxicology testing. I would definitely like to keep doing research.

Please answer the question from the last toxafette PhD-candidate: What was (up to now) the best experience you had while doing your PhD research?

During the design of the study protocol of my human volunteer study, I came up with a new idea to collect data from our volunteers. I wasn't sure if it would work, and it definitely needed some testing before we could include it in the study design. Together with another researcher we started experimenting in the lab and improving the idea to something more concrete. This was very interesting and challenging, as well as a lot of fun when we figured out it might actually work. Having this idea, working it out and reporting the results to others was a really nice experience.

What question would you like to ask the next Toxafette candidate?

My question for the next person is: What is something you would have liked to know about doing a PhD, back when you were still a student?

REGISTRATIE CIE

Voorletters	Achternaam	Datum inschrijving	Datum afloop registratie
J.	Roosendaal	26-01-2023	26-01-2028
C.	Liu	26-01-2023	26-01-2028
J.	Jin	26-01-2023	26-01-2028
R.H.	Mennen	26-01-2023	26-01-2028
V.P.L.	van de Weijgert	26-01-2023	26-01-2028

Especially for all professional toxicologists
looking to renew their registration:

New PET course 'Current Contents in Toxicology'

April 11-12, 2023, Congres
Centrum Woudschoten, Zeist

This new course was given for the first time last year and received a positive evaluation. The course is intended for (registered) toxicologists who would like to keep up to date on the latest developments in the field of toxicology. In 1.5 days, new insights and perspectives in key current topics in toxicology will be presented, while at the same time providing a great opportunity to network with colleagues from different professions in toxicology.

The course consists of 3 parts, namely an afternoon and evening, and the next morning. The first day part will focus on **New Developments in Human Based Models** and the evening program will focus on **Endocrine Disruptors**. The last part in the morning of the second day is entitled **Safe-by-Design**, focusing on the testing for safety of compounds/materials during the development process. For more information, please see this [link](#).



Annual NVT Meeting Toxicology: does one size fit all?

Dear NVT members,

We are happy to invite you to the Annual NVT meeting at 'congrescentrum de Reehorst', Ede on **June 7 and 8**. The date for the 'young scientists' day is June 7th. The 'NVT-member' day, including the annual NVT business meeting, is scheduled for June 8th.

The theme of this year will be: Toxicology: does one size fit all?

Every toxicologist, regardless of whether they are active in academia, industry, or the regulatory field, has had to deal with research results not being applicable to everyone. This can be due to a specific study design or exclusion of certain individuals from participating in research. Even in present day drug development, we see examples of widespread adverse effects after compounds have entered the market, because large groups of the population are being left out in (clinical) studies. Standard research, one size, does not fit all. But what should we do? How can we address this? And what can we do to be more inclusive?

This annual NVT meeting will highlight the progress made and challenges ahead spanning different fields of toxicology. We will focus on selecting the right study design and discuss how toxicology can be more inclusive. We will cover all sizes: from nanoparticles to biomaterials and from personalized medicine to population risk assessment.

Registrations are open and the deadline for submitting abstracts is **April 4th**.

The preliminary program is already available on www.meeting2023.toxicologie.nl.

Best regards,
NVT organizing committee

Nathalie Dierichs, Irene Gosselink, Joyce van der Heijden, Vienna van de Laarschot,
Julia Meerman, Kiri Romano Olmedo, Hans Bouwmeester, Laura Hondebrink, Juliette Legler,
Yvonne Staal, Peter Theunissen



Fun Facts about TCDD's (Theme) History

DID YOU KNOW THAT?

- TCDD stands for Toxicologische Communicatie, Data & Documentatie (or the dioxin-like compound 2,3,7,8-Tetrachlorodibenzo-p-dioxin)
- There used to be only 1 editor until the NVT website was launched in 2000? That year's first TCDD included a call for more editors due to the increase in workload. In December '00, three new editors were announced. At that time the TCDD look liked this (picture 1 – TCDD '00 ed 1).
- The freshly started editorial team worked hard to create a new layout, which was shown in the beginning of 2002 (picture 2 - TCDD '02 ed 2). Since 2014, TCDD editorial team can rely on the outstanding graphic skills from Marleen Mulder at Green Bean Design (picture 3- TCDD '14 ed 1). Content-wise (e.g. rubrieken, topics, working with themes) it remained quite similar to the TCDD format published since '00/'01.
- Because of NVT's 30th anniversary, TCDD editorial team created a special theme for each edition that year. The first one was aligned with the annual meeting (NVT meeting 2009 - Innovative Toxicity Testing). This was the start of a new tradition. With exception of 2015, there was at least 1 theme edition every year.

- Since 2020, each TCDD edition has a theme. As in previous years, theme editions contain articles dedicated to the theme as well as our regular topics (i.e. "rubrieken") that can be either in- or outside the scope of the theme.
- The current editorial team is working on an exciting new publication format which has been approved by the board already. At this moment in time we cannot reveal more information but STAY TUNED, WE ARE SURE YOU WILL LIKE IT!



Picture 1



Picture 2



Picture 3

New puzzle!

All theme topics are listed below in chronological order (year-edition). The theme words shown in bold can be found in the puzzle. Words are shown horizontally, vertically and diagonally and can be spelled backwards too. Words belonging to 1 theme title (e.g. TCDD Beyond Borders) are shown as separate words (not grouped together). Words may partly overlap (e.g. crisis and safety, shown in puzzle as crisisafety). **Cross out all the bold words from the list of theme topics. Use the remaining letters to find the puzzle's solution (extra hint: you can place the letters in chronological order on the dots below). Send your solution to redactie@toxicologie.nl and you can win a prize!**

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

- | | |
|--|---|
| 2009-1: Innovative Toxicity Testing | 2018-2: Food toxicology |
| 2009-2: Toxicologie Internationaal | 2018-3: Green toxicology |
| 2009-3: Toxicologen in het buitenland | 2018-4: Microplastics |
| 2009-4: CRISIS! | 2019-3: Artificial intelligence |
| 2010-3: Geneesmiddelen toxicologie | 2020-1: Animal free safety |
| 2011-1: Drugs in sports | 2020-2: Corona virus |
| 2012-1: Voedselveiligheid (voedsel-veiligheid) | 2020-3: Ecotoxicology 2020 |
| 2012-4: Forensische toxicologie | 2020-4: Covid-19 vaccine special |
| 2013-3: Grenzenloze Toxicologie | 2021-1: Post- BREXIT safety and toxicology |
| 2014-3: Celebrations | 2021-2: Food and feed toxicology |
| 2014-4: Afscheid | 2021-3: EU chemicals strategy |
| 2016-1: Developmental Toxicology | 2021-4: Forever chemicals
(Persistent Organic Pollutants) |
| 2016-2: TCDD Beyond Borders | 2022-1: Inhalation toxicology |
| 2016-3: Pharmaceutical Toxicology | 2022-2: Petroleum toxicology |
| 2016-4: Alternatives to animal experiments | 2022-3: Water |
| 2017-1: Rubberkorrels (rubber-korrels) | 2022-4: Toxic lifestyles |
| 2017-4: Reflecting on the future | 2023-1: AI and Computational Toxicology |
| 2018-1: Mixture toxicology | |

FIND THE PUZZLE'S SOLUTION:

.....,!,,

.....?

.....

Christmas Puzzle Winner: Folke Dorgelo!

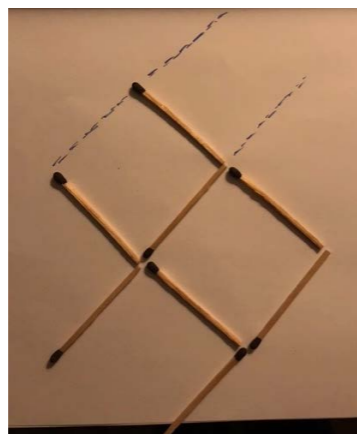
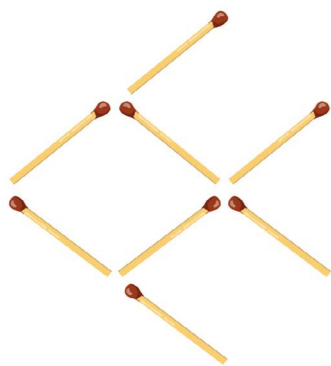


Folke is the winner of the Christmas Puzzle.
Congratulations! The results submitted:

1. Move two matchsticks so the glass is reformed without the coin inside.



2. Can you make the fish swim in the opposite direction by moving only three matchsticks?



3. Move one matchstick to make a correct equation.



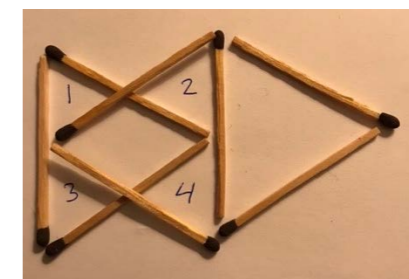
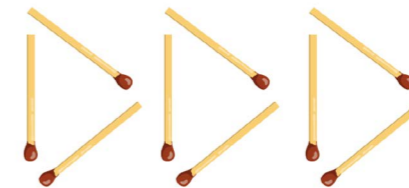
4. Change three matchsticks into six without breaking any of them.



5. Using just six matchsticks, make four equilateral triangles.



6. Move three matchsticks to make four equilateral triangles.



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

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

