Division of Toxicology WUR

Master thesis booklet

Division of Toxicology Wageningen University

If you are you curious about how everyday chemicals - from pesticides to microplastics - affect living organisms at the molecular level and you want to contribute to research that bridges biology, toxicology, and environmental science, then the Division of Toxicology is the place to be! Whether you're interested in molecular biology, environmental health, or toxicology, this is a chance to work on meaningful research with real-world implications. You will gain hands-on experience with cutting-edge techniques and contribute to a growing field that informs public health and policy. An internship with us is a great opportunity to contribute to interdisciplinary research at the intersection of biology, toxicology, and environmental science. You'll gain valuable skills for careers in academia, industry, or regulatory science.

Helix, Building 124, Stippeneng 4, 6708 WE Wageningen

WHAT IS TOXICOLOGY

Toxicology aims to study the potential harmful effects of chemicals on humans and the environment. This is relevant for a broad range of chemicals and materials, for instance:

- chemicals that may occur in our food intentionally or unintentionally (e.g., food additives and contaminants),
- chemicals (pollutants) that are present in the environment and may affect the health of wildlife and ecosystems
- natural plant-, algae-, fungi- or bacterial toxins
- nano- and microplastics
- (new) drug entities,
- Chemicals resulting from food processing (e.g. advanced glycation end products)

OUR RESEARCH IN THE DIVISION OF TOXICOLOGY

Toxicology is undergoing a paradigm shift from routine testing of chemicals to integrating advances in molecular and cell biology, analytical chemistry and bioinformatics to drive a mechanistic understanding of the progression of toxicity events across different levels of biology (cells, tissues, organs and individuals) that lead to adverse health effects in humans and the environment. The toxicology chair group, led by Prof. Dr. Nico van den Brink, develops new tools and strategies to assess the human and environmental health risks of chemicals via hypothesis driven research.

Food toxicology

We quantify the levels of natural toxins in food products to help regulatory agencies gain the mechanistic information they need to make reliable risk assessments and keep food safe.

Advanced *in vitro* models

Traditional toxicity testing of chemicals uses animal tests. We develop and implement advanced human cell models to better understand the action of potentially harmful chemicals.

Environmental toxicology

We investigate the effect of low, yet constant, exposure to contaminants on the environment and its organisms. We are interested in changes to gene expression and telomeres as well as immune function.

Toxicokinetics

We use new tools in tissue culture, computational modelling and analytical chemistry to understand and predict the extent to which chemicals accumulate in target organs like the brain, liver and kidney.

A central theme of our research activities is to explore the mechanisms behind toxicity, and where possible adhere to the “3R concept” of reduce, refine and replace animals in toxicity studies. Therefore, we use:

- cell based methods. We culture and expose cells and subcellular fractions obtained from organs in humans and animals, such as liver, kidney, brain, intestinal tissue, and gills;
- induced pluripotent and adult stem cells differentiated into heart, liver, and intestinal tissue;
- intestinal microbiota;
- computational tools, including physiologically based kinetic (PBK)modelling and quantitative structure activity relationships (QSARs);
- the nematode *Caenorhabditis elegans* and the fruit fly, *Drosophila melanogaster*;
- in addition, we perform (field) studies with (small) animals and birds.

To study the effects of chemicals, the department has excellent research infrastructure:

- cell culture facilities;
- microscopes, with access to advanced confocal microscopy;
- qPCR, ELISA, flow cytometry and other molecular biology equipment;
- microfluidic ‘organ-on-chip’ devices;
- advanced analytical chemistry facilities (LC-MS quadrupole and TOF, GC-MS, UPLC-UV, and fluorescence);
- mesocosms and other experimental setups.

THESIS IN THE DIVISION OF TOXICOLOGY

Thesis topics at the Division of Toxicology are divided into 2 major themes, human or environmental toxicology. We do work together as a team, and many project cross the themes. For arranging an MSc thesis subject, you can contact office.tox@wur.nl. For inspiration of the projects we have on offer, please see the research topics listed below. Please note that apart from the projects listed, there are also possibilities for performing a desk-based study on, for example, a risk assessment on a specific (group) of chemicals based on literature studies at the Toxicology department.

If you are interested in potential topics focusing on human toxicology, go to pages 6-13
For those interested in environmental toxicology, go to page 14 and beyond

TOX-H1: The Virtual Human Platform for Safety Assessment

Supervisors: Nynke Kramer

As a part of the Virtual Human Platform 4 Safety (VHP4Safety) project, this PhD project aims to reduce animal studies for safety testing of nephrotoxicants. Safety testing relies heavily on animal studies, even though animal tests are poor at identifying (doses) of chemicals causing toxicity in the kidney of humans. New approach methodologies (NAMs), including in vitro toxicity assays with human proximal tubule cells and computational tools like physiologically based kinetic (PBK) modelling have the potential to better characterise the hazards and risks of developing nephrotoxicity after of chemical exposure. In this project, different in vitro human renal proximal tubule cell systems will be assessed for their potential to form tight monolayers and their expression and activity kidney-relevant metabolising enzymes and xenobiotic transporters, especially the organic cation transporter 2 (OCT2). The in vitro model deemed to have sufficient transporter and enzyme activity will be used to assess the clearance and nephrotoxicity of cationic pesticides, like mepiquat. These clearance values will be used to parameterise PBK models to evaluate differences in renal clearance and nephrotoxic potency of pesticides in between animals and humans, between healthy and diseased humans, and between men and women.

A thesis project could be: Comparing the expression of renal markers and/or the permeability of OCT2 substrates in renal proximal tubule cultured under varying culture conditions including as monolayers, in transwell systems, and/or under flow.

TOX-H2: Maintaining concentrations of 'difficult' test chemicals in *in vitro* toxicity assays constant to improve quantitative in vitro in vivo extrapolations (QIVIVE).

Supervisor: Alexandros Sotiriou, Sebas Wesseling; Supervisors: Nynke Kramer, Chiel Jonker (Utrecht University)

The nominal concentration, i.e. the theoretical concentration based on amount of test chemical added to culture medium, is generally used to express concentration-effect relationships in in vitro toxicity tests. However, for instable, volatile, lipophilic, and highly plasma protein bound chemicals, the nominal concentration does not represent the concentration responsible for the observed effects at the target site in cells. In this project, we develop tools that control for the degradation, evaporation, and binding of chemicals to the in vitro system setup of these chemicals. These tools include exposing cells in sealed glass vials and microtiter plates dosed through polymers loaded with test chemicals (i.e. partition-controlled dosing). A decision tree will be evaluated to allow researchers to determine when to use these dosing tools based on the properties of the test chemical and in vitro test system. Research techniques used in this project include computation modelling (PBPK and QSARs), analytical chemistry and cell culture.

A thesis project could be: Analytically measuring the free concentration and accumulation of difficult to test chemicals, such as phthalates and bisphenols found in plastics, in human liver and mouse embryonic stem cells in vitro over time and/or comparing these concentrations with those predicted by computational models.

TOX-H3 Developing an ontology approach to assess toxicological risk of chemical exposure in humans without animal testing (ONTOX)

PhD: Sylvia Adam; Supervisors: Nynke Kramer, Hans Bouwmeester

The ONTOX project is an EU funded, multimillion Euros research project where 19 research organisations across Europe aim to deliver a generic test strategy to predict systemic repeated dose toxicity without using animal tests. This strategy should be applicable to any type of chemical, including pharmaceuticals, cosmetics ingredients, and foodborne contaminants, to predict the hazard and risk of chemical-induced liver steatosis and cholestasis, tubular necrosis and crystallopathy in the kidney, and neural tube closure and cognitive function defects in the unborn child. The different sources of toxicity information to be integrated in the strategy include computational models, such as environmental fate models predicting the level to which humans are likely to be exposed, quantitative structure activity relationships (QSARs) relating a chemical structure to a toxic effect, physiologically based pharmacokinetic (PBPK) models to assess the extent to which a chemical reaches a target organ in the body once humans are exposed, and systems biology modelling to predict how much change at a molecular target, such as a receptor in the brain, is required to cause disease. Artificial intelligence (AI) will be integrated into these computational models to improve their predictivity. Other sources of information to be integrated into the framework include advanced in vitro cell models mimicking the physiology of the liver, the kidney, and developing brain on which chemicals are tested to ascertain the extent to which these chemicals are absorbed, distributed, metabolised, excreted and cause molecular changes associated with repeat-dose toxicity. At WUR, the focus is on developing chemical distribution models to determine the concentration of a chemical reaching the molecular target in (1) in vitro cell-based toxicity assays and (2) a tissue in the body given the nominal dose. These distribution models simulate the movement of the chemical in time through a system, such as an in vitro cell assay or the human body. Long-lived in vitro assays with human liver, brain and kidney cell lines will be exposed to case study chemicals in repeat-dose experiments to determine the uptake, clearance, and toxicity of these chemicals, which is required for input into PBK models. Research techniques used in this project therefore include cell culture, molecular biology (e.g., qPCR), analytical chemistry and computational modelling

A thesis project could be: Analytically measure the concentration of different azole fungicides, perfluorinated alkylated substances (PFAS) or MCPD in plastic, medium and cells in in vitro liver steatosis, in vitro neurotoxicity, or in vitro kidney chrystallopathy models, respectively. Azole fungicides are widely used and may cause liver steatosis. PFAS exposure in utero is associated with neurodevelopmental defects. MCPD is produced when foods are treated at high temperatures with acids, which is done to make food more digestible. There are indications that they can cause kidney injury. Knowing the exact concentration in cells can be used to do quantitative in vitro to in vivo extrapolation (QIVIVE) to assess the dose at which humans will experience these toxicities.

TOX-H4: The assessment of the health risks of mixtures of flavanoid-like chemicals in botanical supplements using new approach methodologies (NAMs)

PhD: Xuan Zhang; Supervisors: Nynke Kramer

Flavanoids are a class of polyphenolic secondary metabolites found in plants and therefore commonly consumed through our diet. They are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. There are thousands of variants of flavanoids, including anthocyanins and flavanolignans. They are thought to have anti-oxidant properties and may therefore be considered nutraceuticals. However, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) in the human body for most of these flavanoids is unknown. Flavanoids are also endocrine disruptors and may cause developmental toxicity. To test for the toxicity of all these flavanoids is infeasible. It would take too much resources and importantly too many lab animals. Therefore, it is important to develop and employ new approach methodologies (NAMs) to assess the ADMET of flavanoids and obtain a mechanistic understanding of how the chemical structure drives the ADMET of flavanoids. This project is divided into four parts: (1) assess how the chemical structure affects rat and human gut bioavailability of flavanolignans in silymarin, a milk thistle supplement, and anthocyanins, natural food colourings, using different in vitro gut permeability assays, (2) study the rat and human clearance and metabolic pathway of silymarin constituents and anthocyanins by gut microbiota, enterocytes, hepatocytes and/or kidney proximal tubule, (3) compare the toxic potency of anthocyanins and/or silymarin constituents and/or their metabolites in in vitro toxicity assays testing for inhibition of the thyroid hormone transporter protein in developing brain, MCT8, embryotoxicity and oxidative stress, and (4) apply quantitative in vitro to in vivo extrapolation (QIVIVE) using a read-across-physiologically based kinetic (PBK) modelling approach to derive benchmark dose levels (BMDL) for rat and human developmental toxicity of silymarin and individual silymarin constituents and/or anthocyanins.

A thesis project could be: Analytically assess the bioavailability of anthocyanins or silymarin constituents in in vitro permeability and metabolism assays, and develop structure activity (SAR) models to predict the toxicokinetics and estrogenicity of the flavonoids and flavonolignans. Techniques to work with include cell culture, analytical chemistry, and computational modelling.

TOX-H5: ADME4NGRA: Implementing the EFSA NAMs roadmap through advancing toxicokinetic knowledge in chemical risk assessment

PhD: Feihan Lin; Supervisor: Nynke Krame, Jingxuan Wang (WFSR)

Hazard characterisation in traditional risk assessment of food and feed ingredients is generally based on sub-chronic and chronic oral toxicity studies on animals. New approach methodologies (NAMs), including assays based on in vitro and in silico models, provide more mechanistic tools to study the hazard and risk of chemicals in specific species and human populations. Case studies are need to illustrate how advanced in vitro models assessing the absorption, distribution, metabolism, and excretion (ADME) potential of food ingredients and contaminants, can and should be used to parameterise generic physiologically based kinetic (PBK) models to perform quantitative in vitro to in vivo extrapolation (QIVIVE) and assess inter and intra-species variability in toxicokinetic. This project, funded by European Food Safety Authority (EFSA) and involving 10 institutions across Europe, aims to provide a list of in vitro and in silico ADME models and guidance for using these models. At WUR, the focus is on developing and validating in vitro intestinal models for parameterising PBK models to identify potentially hazardous substances to humans based on their ADME profiles. The extent to which foodborne chemicals are absorbed into the body is determined by the flux of the chemical across the intestinal epithelium, which is dependent on not only passive and facilitated diffusion across the barrier, but also pre-systemic metabolism by microbiota and enterocytes, as well as active transport in and out of enterocytes transport protein. Generic PBK models largely ignore possible species and interindividual differences in transporter activity and metabolic capacity which drive variability in toxicity. For this, we developed state-of-the-art in vitro intestinal systems using adult stem cells derived from both human and rat intestines. These in vitro models will be used to study phase I and phase II intestinal metabolism and transport of chemicals found in food ingredients and contaminants. The traditional permeability models, such as those based on Caco-2 cells, will also be assessed.

A thesis project could be: application of the in vitro stem cell based intestinal models to study the intestinal transport and metabolism of case study chemicals, including cyanotoxin microcystin, plasticizer 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH), immunosuppression drug tacrolimus, food ingredient resveratrol and mixture of pesticides, and/or integrating these in vitro derived data in PBK models to better predict the variability in blood and tissue concentrations of chemicals in human or rats for toxicological risk assessment.

TOX- H6: Development of immunocompetent liver toxicity models to unravel the role of KCs in hepatotoxicity

PhD: Gijs van Slobbe, Supervisors: Coen Govers (ASG-CBI), Mathias Busch, Hans Bouwmeester

Current hepatoxicity adverse outcome pathways (AOPs) and hepatotoxicity testing strategies do not address immunological key events (KEs). Kupffer cells (KCs), the major tissue-resident macrophage population in the liver, play a crucial role in modulating liver adverse outcomes (AOs) including liver injury, cholestasis, and steatosis. Therefore, this PhD project focusses on the development of immunocompetent liver toxicity models by inclusion of KCs in hepatocyte co-cultures. Induced pluripotent stem cell (iPSC)-derived KCs (iKCs) will be generated and subsequently implemented in co-culture models with HepaRG cells to assess hepatotoxicity. Main iKC-driven endpoints that will be assessed upon exposure to relevant chemicals include hepatocyte cytotoxicity, fatty acid accumulation and bile acid transport, which respectively address the liver AOs of liver injury, steatosis, and cholestasis. Successful development of these models targets the knowledge gap of the role of the immune system in liver toxicity and could potentially lead to implementation of these models as new approach methodologies (NAMs) for next generation chemical safety assessment. The selection of testing chemicals and toxicological endpoints will reflect chemicals from different regulatory domains (i.e. food, drugs, and environmental contaminants.

A thesis project could be: Development of a THP-1: HepaRG coculture model to assess inflammatory-driven modulation of either steatosis or cholestasis. This includes the culturing of these cells, readouts can include cytotoxicity, Rt-qPCR readouts, and LC-MS based detection of bile acids and or fatty acids.

TOX- H7: Assess the uptake and toxicity of micro- and nanoplastics

PhD: Rizal Makarim, Supervisors: Hans Bouwmeester, Mathias Busch

The exponential increase in the production/use of plastic translates into a parallel increase of environmental plastic-waste that is continuously degraded into micro and nanoplastics. These tiny plastic particles have been detected in food and drinks, but also human tissue samples. Information on the effect of micro and nanoplastics on human health is still preliminary, which makes it currently very difficult to perform a risk assessment. In this project, we use advanced in vitro models of the gastrointestinal barrier to study the intestinal uptake and toxicity of micro and nanoplastics, and translate the in vitro results obtained to the in vivo human situation.

A thesis project could be: Use of intestinal and /or immune cells which will be exposed to different types of nano and microplastics. Readouts could be cytotoxicity, gene expression, cytokine release and confocal microscopy. Analytical techniques used could be dynamic light scattering to characterize size of the nano and microplastics.

TOX- H8: Hazard characterization of leachable chemicals present in plastics

PhD: Elise van der Koogh; Supervisors: Hans Bouwmeester, Mathias Busch, Nynke Kramer

Plastics contain numerous chemicals that can leach into surrounding environment, including food and water. Ingestion is therefore a major human exposure route, yet the toxicological effects of these complex chemical mixtures remain poorly understood. The intestinal epithelium plays a crucial role in absorption, immune defense, and homeostasis, and its disruption has been linked to systemic inflammation and disease. While some plastic-associated chemicals (e.g. bisphenols, phthalates, and PFASs) are known to cause endocrine disruption, genotoxicity, or inflammation, hazard data for most leachable compounds, especially their mixture effects, are lacking.

This PhD project investigates the chemical composition and toxicological effects of leachates from commonly used consumer plastics. Leachate profiles will be assessed through untargeted GC-MS/MS screening. Endocrine-disruptive activity will be assessed using CALUX reporter cell lines, while advanced *in vitro* intestinal models will be used to evaluate genotoxicity, inflammation, barrier integrity, and bioavailability. Physiologically based kinetic (PBK) modelling will be applied to estimate internal exposure and support human health risk assessment. By linking chemical mixture profiles to biological responses, this project will provide human-relevant insights into the hazards of plastic leachates and inform safer product design.

A thesis project could be: Evaluate the endocrine-disrupting potential of plastic leachates using CALUX reporter cell lines, including cytotoxicity assessment (e.g. WST-1, alamarBlue, LDH assays) and linking GC-MS/MS chemical profiles of leachates to observed biological activities. The project may also involve the (co-)culturing Caco-2 cells and assessing inflammation-related cytokine release and gene expression.

TOX-H9: Multiomics analysis of advanced glycation end products in organotypic in vitro models

PhD candidate: Haomiao Wang; Supervisor: Nynke Kramer

Advanced glycation end products (AGEs) are a group of compounds that form when sugars react with proteins, lipids, or nucleic acids without the involvement of enzymes. They can naturally occur in the body as a byproduct of metabolism, but they can also be formed during food processing, especially when high-heat cooking methods like grilling, frying, or roasting are used. Accumulating evidence suggests that high levels of AGEs in the body may contribute to various health problems, including inflammation, oxidative stress, and the development of chronic diseases such as chronic kidney injury, diabetes and neurodegenerative diseases such as Alzheimer's Disease. The molecular mechanisms by which these chemicals induce neurotoxicity and nephrotoxicity, however, is still largely unknown. With the advent of new 'omics' technologies this gap in knowledge can be bridged. The aim of this project is to test for the natural levels of AGEs in neuronal and kidney cells in vivo and in vitro and expose these cells in vitro to AGEs with and without the presence of anti-oxidants to obtain dose response curves for the level of modified DNA, amino acids and proteins, which can subsequently be used in a quantitative in vitro to in vivo extrapolation (QIVIVE) approach to perform risk assessments.

A thesis project could be: Apply the advanced in vitro intestinal, blood brain barrier, neuronal and kidney models to study the transport of AGEs across tissue barriers; develop an omics approach to identifying DNA modifications in neuronal cells in vitro.

TOX-H10: High resolution mass spectrometry methods to help assess the health risk of bee pollen supplements.

PhD candidate: Susannah Heeren; Supervisor: Nynke Kramer, Laura Righetti (Analytical Chemistry)

Bee pollen supplements are a type of natural supplements. They are an agglutination of flower pollen collected by the honey bee. The pollen are mixed thee bee's mouth secretion and nectar, to secure safe arrival at the beehive. Once the bee gets to the bee hive, the pollen are strapped of their legs by pollen traps and collected for human consumption. Over the years this type of supplement has grown in popularity thanks to their nutritional and medical properties. However, just because they are natural, it does not mean they are safe. There have been numerous studies showing that bee pollen are often contaminated with hazardous compounds such as pyrrolizidine alkaloids, mycotoxins, and pesticides. However, these studies often focus on only one class of contaminants and neglect the others. That is why we are focusing on exploring the entire bee pollen exposome and metabolome. A non-targeted High Resolution Mass spectrometry method has been developed to chemical characterize the bee pollen. With this method a variety of pesticides and pyrrolizidine alkaloids were found. To further investigate these two groups and possible mixture effects between them we need to know which ones and in what concentrations they are present in the sample. Therefore we need to analyze the bee pollen samples via targeted LC-MS analysis for both the pesticides and pyrrolizidine alkaloids. With that data we can then study possible mixture effects via exposure assessments.

A thesis project could be: Targeted LC-MS analysis of pesticides and pyrrolizidine alkaloids in bee pollen and studying possible synergistic and/or antagonistic effects between pyrrolizidine alkaloids and pesticides through *in vitro* assays such as micro-ames and/or comet assay.

TOX-H11 Investigating inflammation-derived modulation of toxicokinetics *in vitro*

Supervisor: Mathias Busch

Chronic or recurring inflammation is involved in a large number of human diseases, affecting many toxicologically relevant organs, such as the intestine, lung, liver, kidney, nervous system or skin. Pre-existing inflammatory conditions can affect the toxicodynamics and -kinetics of drugs, chemicals and environmental pollutants e.g. via increased permeability of the gut, lung, skin or the blood-brain-barrier, decreased metabolic capacity of the liver, or reduced filtration efficiency in the kidney. While the effects of inflammation on toxicodynamics and – kinetics can be modelled *in vitro* for the liver, data on in vitro models of the intestine, kidney, lung, skin and brain is still scarce.

A thesis project could be: Investigating changes in toxicokinetics under inflammatory conditions in *in vitro* models of different organs. Methods: Cell culture, ELISA, qPCR.

TOX-H12 Toxicological effects of food borne contaminants on in vitro models of the inflamed liver

PhD candidate: Thorieq Ali; Supervisors: Hans Bouwmeester, Mathias Busch

Current hepatotoxicity adverse outcome pathways (AOPs) and testing strategies do not address immunological key events (KEs), while regulatory authorities have identified the lack of immunocompetence in current toxicological testing approaches as an emerging knowledge gap. Chronic, low-grade liver inflammation is known to affect the functioning of hepatic metabolic enzymes (i.e., Phase I cytochrome P450 enzymes and Phase II enzymes), thereby influencing the toxicity of foodborne chemicals that require bioactivation by these pathways. A well-known example is aflatoxin B1 (AFB1), a mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus, commonly found in agricultural products such as maize, peanuts, and other nuts when storage conditions are poor. This toxin can cause acute liver injury and, under conditions of chronic exposure, is linked to carcinogenesis within adverse outcome pathways. It is therefore crucial to develop efficient techniques for evaluating the influence of foodborne contaminants on inflamed liver tissues to guarantee food safety and consumer health. In this project, different in vitro models and approaches will be applied to establish effective and reliable strategies to evaluate the effects of foodborne contaminants in inflamed liver tissue, with a particular emphasis on genotoxic outcomes.

A thesis project could be: Investigating ROS production and DNA damage in inflamed liver models after exposure to foodborne contaminants. Techniques could include cell culture, ELISA, qPCR, comet assay, DCFH-DA assay.

TOX-H13 INTENTION: Bioplastic derived micro- and nanoparticles affect the developing intestine of infants

PhD candidate: Zoe Berkers; Supervisors: Hans Bouwmeester, Mathias Busch

Fossil-fuel-based plastics are being used in increasing quantities in everyday life. Biopolymer-based plastics are emerging as a more environmentally friendly alternative. In the environment, large pieces of plastic degrade into micro- and nanoplastics (MNPs). While bioplastics can be efficiently decomposed under industrial conditions, this efficiency may not be replicated in natural environments, leading to the presence of biopolymer MNPs in the environment. As a result, human exposure to biopolymer MNPs is likely. Concerns have already been raised about the health impacts of fossil-based MNPs, and emerging evidence suggests that biobased MNPs may have similar effects on human health. Young children are at an increased risk of exposure, as they come into contact with MNPs from plastics through various sources such as food, toys, feeding bottles, and playgrounds. Infants are particularly vulnerable to ingested MNPs due to the physiological, immunological, and microbial changes that occur in the gastrointestinal tract after birth, especially
when the introduction of solid food significantly alters the composition and diversity of the microbiota. Adverse impacts on intestinal health and microbiota during early life could have long-term consequences, potentially increasing the risk of conditions such as allergies and other diseases. In this project, we aim to develop an advanced model using stem cells derived from biopsies taken from children's intestines, which will be grown into a miniaturized, three-dimensional model of the intestine. This "gut-on-chip" model will allow the apical (luminal) side of the intestinal cell monolayers to be maintained under anaerobic conditions, closely mimicking the interactions between the microbiota, intestine, as observed in humans. The intestinal monolayers will be exposed to MNPs to assess their potential adverse effects on gut health, including their impact on the microbiota, without the need for experimental animals or invasive sampling in children.

A thesis project could be: Exposure of intestinal cell monolayers to microplastics with subsequent toxicological assessment of effects in the presence or absence of microbiome-derived proteins. Another project could be to asses effects of microplastics on the growth of microbial communities associated with the infant gut.

TOX-H14 INTENTION: Modelling Vitamin A Exposure

Supervisors: Nynke Kramer and Alida Melse (Division of Human Nutrition and Health)

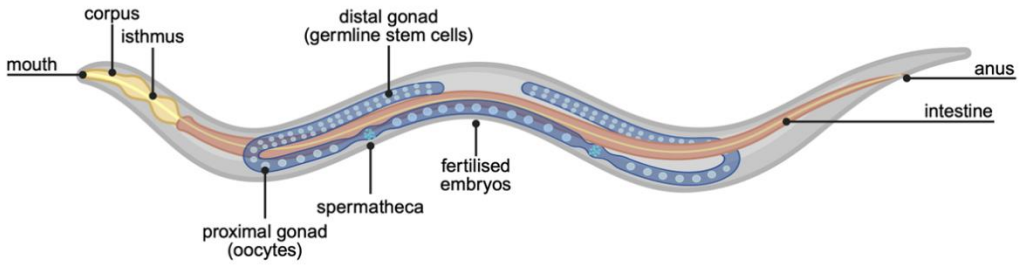
Vitamin A deficiency and hypervitaminosis A remain significant and co-existing public health concerns worldwide, particularly in regions where supplementation programs, dietary variability, and infection interact to influence vitamin A metabolism. Current approaches to assessing vitamin A status rely largely on biomonitoring markers such as serum retinol, which are tightly homeostatically controlled and therefore poorly reflect total body stores or the risk of deficiency and toxicity. To address this limitation, this study proposes a the development of a physiologically based pharmacokinetic (PBPK) model for vitamin A that mechanistically represents absorption, transport, hepatic storage, mobilisation, and tissue distribution of retinoids across key compartments. The aim is to consider the inclusion of retinol-binding protein dynamics, and dietary intake variability to simulate interindividual differences arising from age, nutritional status, infection, body composition, and genetic factors affecting retinoid metabolism. Using biomonitoring data from population studies, model outputs will be evaluated to link observable biomarkers with underlying liver vitamin A stores, enabling improved interpretation of surveillance data. By capturing the nonlinear dynamics of storage and mobilisation, the PBPK framework will allow evaluation of both deficiency risk and the potential for toxicity under varying intake scenarios, including high-dose supplementation programs and food fortification strategies.

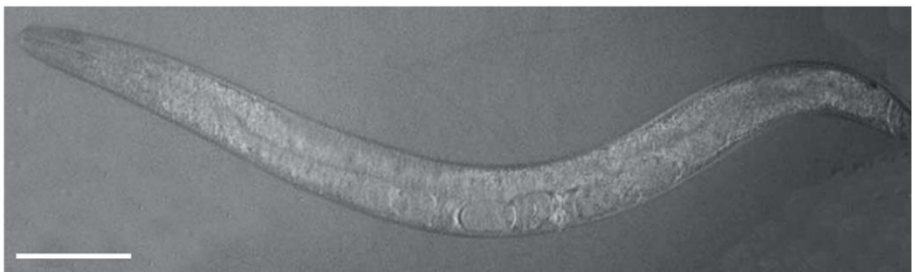
TOX-E1: Working with *Caenorhabditis elegans*

Supervisor: Samantha Hughes

The 1mm long nematode worm *Caenorhabditis elegans* is remarkable in that it combines the simplicity of cell culture with the complexity of a multi-tissue organism. With a well-characterised life history, short lifespan of 3-4 weeks and a high reproductive output, *C. elegans* offers unique advantages; 1) they can be bred cheaply, efficiently, and quickly in the laboratory; 2) the genome has been fully sequenced, showing strong genetic homology to mammals with conserved metabolic, neurological and signalling pathways; 3) there is a vast molecular toolbox available, allowing for rapid analysis at the molecular level and genetic manipulation; 4) the transparency of the nematode provides opportunities for real-time imaging using fluorescent reporters and dyes. Together, these features have contributed to establishing *C. elegans* as a whole-organism model in the fields of neurobiology, aging, modelling of human diseases (e.g., neurodegeneration), as well as in toxicological research. While *C. elegans* cannot fully replicate all the processes required to predict the effects of compounds in humans, its genetic, organ and functional homology to other *in vivo* models makes it a relevant whole-organism system for predicting adverse outcomes. We use *C. elegans* to explore the mechanisms that underly the adverse outcomes due to exposure to environmental toxins. These compounds include pesticides, pharmaceuticals, microplastics and persistent organic pollutants like the PFAS family of chemicals.

Potential thesis projects include: Exploring how pesticides induce oxidative stress that leads to neurodegenerative disease?; How do mixtures of chemicals impact development and reproduction? What is the impact of exposure to chemicals "*in utero*" to long term survival? What are the effects of chemical exposure across several generations?





TOX-E2: Developing a generic PBK model for assessing toxicity in birds

PhD: Thitirat Kanokjiraporn; supervisors: Nico van den Brink, Nynke Kramer

This project aims to model the toxicokinetics of environmental contaminants in various bird species using New Approach Methodologies (NAMs). These include in vitro assays and computational models like physiologically based kinetic (PBK) models. The in vitro toxicity assays with bird liver will be conducted to compare xenobiotic clearance and CYP activity across bird species, which will be used to parameterize PBK models. The resulting model will be employed to assess chemical toxicity in birds, with the goal of developing a reliable tool for environmental risk assessment that reduces or replaces laboratory animal testing, thereby supporting more ethical and effective conservation and regulatory decisions.

A potential thesis project could be: Investigating the activities of human orthologs in the liver microsomes of bird species using human CYP prototype substrates.

Tox-E3: Telomere length dynamics: what beyond aging?

Postdoc: Matteo Schiavinato, supervisors: Samantha Hughes, Nico van den Brink

Aging is a universal biological process that shapes physiology, life history and behaviour of, almost, all organisms. It's not just about passing years, but rather about cellular and molecular processes. Telomeres for example, the protective caps at the ends of chromosomes, shorten according to age, but oxidative stress, such as that induced by urban pollution, can accelerate their erosion. Telomere length has become a biomarker of individual condition and has been associated not only to survival and reproduction, but short telomere has also been associated to behavioural traits such as boldness or risk-taking. Urban animals, living in chronic exposure to pollutants, artificial light, and noise, indeed often behave differently from their rural counterparts. These differences may reflect both evolutionary adaptations and molecular mechanisms: the structure of telomere, depending by its length in fact, can modulate genes expression, potentially linking environmental stress to behaviour through epigenetic pathways. Field research on wild birds, combined with controlled experiments in model species and cell systems, allow us to brings together theoretical biology with molecular biology, behavioural ecology and neurophysiology, aiming to address one of the most fundamental questions in biology: how environment influence mechanisms of aging and the way in which organisms navigate life?

Potential thesis projects range from fieldwork on wild passerine birds, to laboratory-based studies on model organisms, to in vitro molecular experiments.

Tox-E4: Urban Stressors on Avian Immune Defenses

PhD: Shivani Ronanki, Supervisor: Nico van den Brink

The aim of this project is to investigate how low dose, chronic exposure to pollution influences the immune system during early developmental stages in wild birds. We assess both the baseline immune competence as well as the ability to mount an immune response using Poly I:C, a synthetic double-stranded RNA that mimics a viral infection. Additionally, we investigate how diet quality particularly the balance between omega 3 and omega 6 polyunsaturated fatty acids (PUFAs) interacts with the immune system, due to their contrasting effects on inflammation. By integrating these approaches, the study aims to reveal how urban environmental heterogeneity and nutritional factors jointly shape immune regulation. The research can help predict epidemiological outcomes of urban pollution in wild birds.

Tox-E5: Pesticide residues in wild birds

PhD: Aafke Saarloos, Supervisors: Nico van den Brink, Paul van den Brink

Birds that live in or near agricultural areas are likely to be exposed to pesticides. However, up till now, it remains largely unknown to which currently-used pesticides birds are exposed and to what extent. The aim of the current project is to get insight into how pesticide residues may potentially accumulate in wild birds. We study for example whether a bird's diet influences the amount and type of pesticides detected, how different agricultural practices affect the presence of pesticide residues in soil, and whether birds found dead (potentially unhealthy) have higher pesticide loads than birds shot by hunters (potentially healthy). To address these type of questions, we collect a variety of samples ranging from soil samples and food items, to livers and other tissues of birds. The samples are analyzed for the presence of pesticides by making use of the highly sensitive analytical technique LC-MS/MS.

As a thesis student, you will have the opportunity to develop your laboratory and analytical skills, contribute to data processing and interpretation, and explore the current black box of potential pesticide exposure in birds and their surroundings.

Tox-E6: Insect specific *in vitro* assay development

PhD: Rebeka Darmati; supervisors: Nico van den Brink, Samantha Hughes, Bruno Campos (Unilever)

The increasing number of chemicals that enter the environment presents a very serious threat to the wellbeing of humans and ecosystems alike. A major protection goal of Environmental Risk Assessment (ERA) are invertebrate species that play an unreplaceable role in the functioning of the ecosystem. However, current ERA protocols are limited to assessing only apical endpoints such as mortality, overlooking more subtle adverse effects on a molecular level leading to underestimation of risk. Due to the highly conserved molecular pathways between species, pesticides often harm non-target organisms such as pollinators, thereby damaging the entire ecosystem. In the scope of Next Generation Environmental Risk Assessment (NGERA), there has been more focus on the adverse effects at lower levels of biological organisation that may lead to adverse outcomes. Adverse Outcome Pathway (AOP) is a conceptual framework developed to better understand mechanisms underlying toxic effects. AOP starts with a Molecular Initiating Event following well described Key Events at all levels of biological organisation, finally leading to an Adverse Outcome. This project aims to provide a proof of concept for Ecdysone receptor agonism AOP. Ecdysone is the hormone responsible for insect development and metamorphosis, but it is also the basis for many pesticide derivatives. Based on the AOP and in vitro assay will be developed to aid the early screening of chemicals.

Potential MSc thesis projects:

· Investigating cross-species sensitivity to pesticides using insect cell lines

· Studying effects of pesticide exposure on the development of fruit flies (Drosophila melanogaster)

· Exploring internal concentrations of ecdysone and insecticides in fruit flies.

Tox-E7: Evaluating the risk of contaminants mixtures to black-tailed godwits

PhD: Barbara Righetto Supervisors: David Gomez Blanco, Nico van den Brink

The main goal of this project is to investigate the risks that contaminants found in the environment pose to a migratory bird species, the black tailed godwit. To do that, we measure the concentration of current use pesticides and other chemicals in environmental matrices, such as soil, earthworms, rice kernels and blood from the godwits. We also use *in silico* tools to try predicting the potential of the chemicals we detect in our samples to cause oxidative stress and endocrine disruption, and we also measure biomarkers in the blood of godwits to check if our predictions are accurate. The end goal of this study is to contribute to the development of tools that can make risk assessment for migratory animals more realistic.

Potential thesis topics: for example, on optimizing methods for the quantification of different chemicals or *in vitro* studies to investigate their effects. But other ideas that fit within the overall goal of the project can also be discussed

Tox-E8: Early Environmental Safety

PhD: Nick van Sabben; supervisors: Nico van den Brink, Sanne van den Berg

In this project we look at the non-target species of pesticides. In modern agriculture we use pesticides to protect our crops, but non-target species are also exposed to these pesticides. Interestingly, some of the non-target species can tolerate very high concentrations of pesticides, while others die from exposure to low concentrations of the same pesticides. Our goal is to better understand the (biochemical) mechanisms that drive these differences in sensitivity. To do this, we examine the steps that between pesticide exposure and the adverse outcome in an organism. These steps can be divided in two components: what the organism does to the chemical (toxicokinetics) and what the chemical does to the organism (toxicodynamics). This part of the project focusses on the toxicodynamics. This includes the interaction with the target site, how well the organism can repair damage and how much damage the organism can take and still survive. A better understanding of the drivers of sensitivity to pesticides will help in developing improved tests systems for newly developed pesticides and help with the design of new compounds that minimize harm to non-target species.

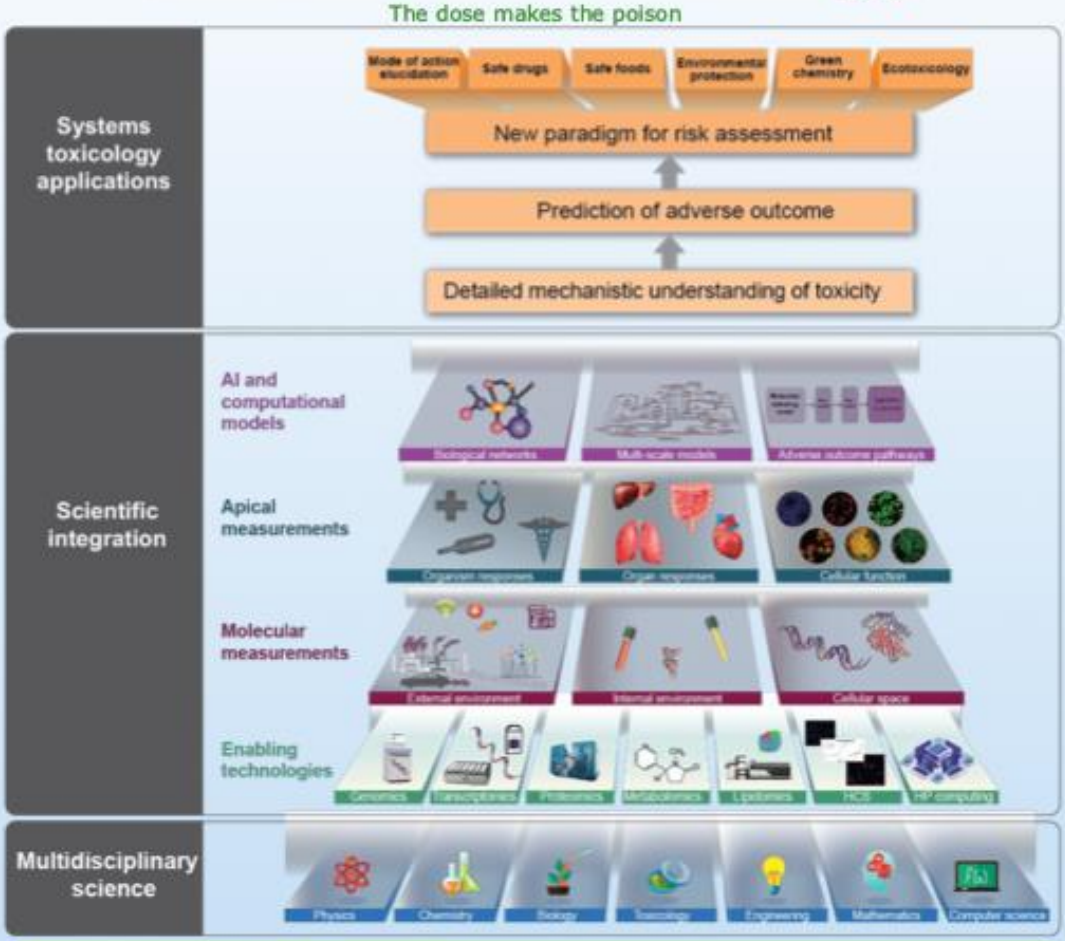
Possible thesis topics:

Analysis of difference in pesticide-target site interaction between species

Measuring key events, such as acetylcholine accumulation, in different species

Division of Toxicology

The dose makes the poison



Human toxicology

- Translation of the in vitro data to the in vivo situation, which can be obtained with help of PBBK modelling
- Focused on developmental toxicity, endocrine disruption, neurotoxicity and on the development of in vitro models for safety testing of nanoparticles
- Mode of action of food-borne (natural) toxins
- The role gut microbiota in the toxicity of food-borne chemicals

Prof. Hans Bouwmeester Dr. Nynke Kramer Dr. Mathias Busch

Environmental toxicology

- Effects of low, chronic exposure of organisms to contaminants is studied
- Focus on the immune system, shortening of telomeres and the occurrence of resistant genes in small mammals
- Effects of nanomaterials on soil invertebrates, and the occurrence of chemicals in Antarctic organisms

Dr. Nico van den Brink Dr. Samantha Hughes

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