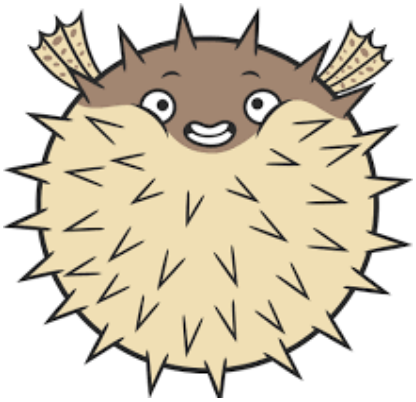
Division of toxicology WUR

Master thesis booklet



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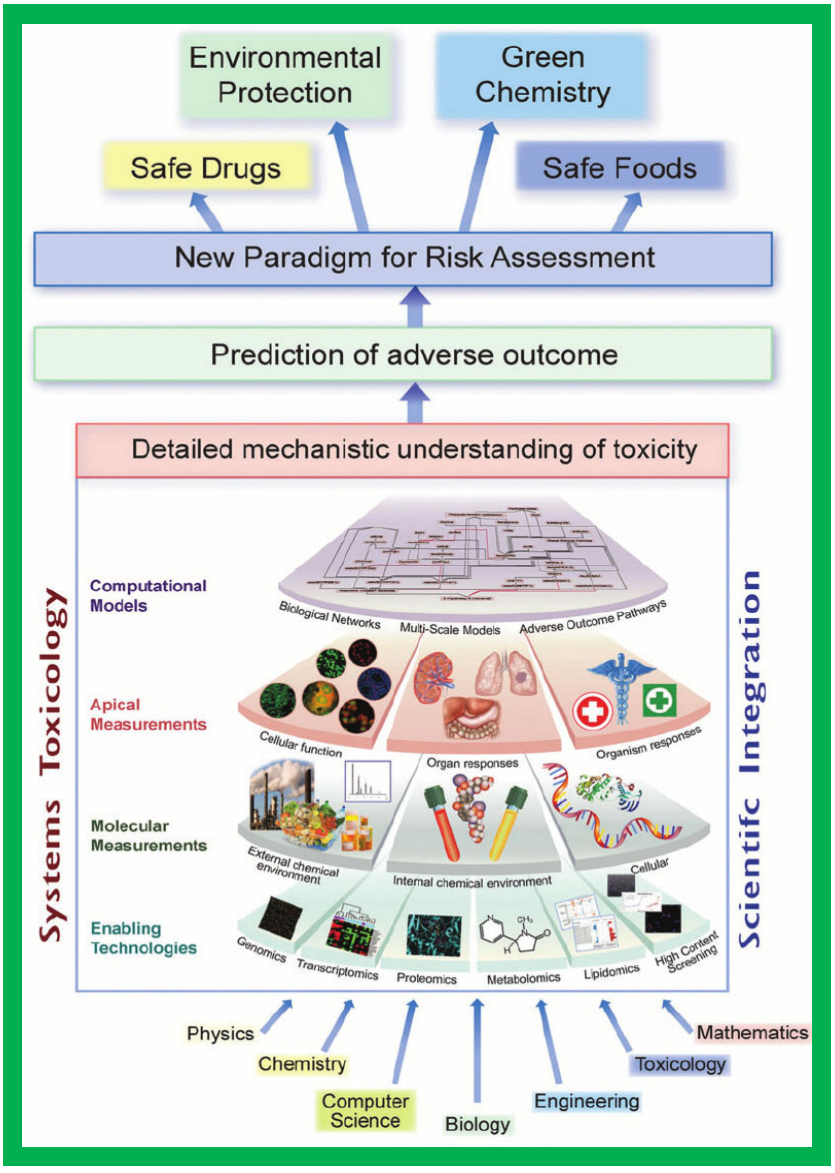
WHAT IS TOXICOLOGY?

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)

Toxicology is an interdisciplinary field bridging medical, biological and chemical sciences.



As a toxicologist you will plan and carry out desk, laboratory and field studies to assess the possible hazards of chemicals as well as the risks of developing adverse health effects after a chemical exposure, both for humans and in the environment. You will consider the potential of modern stat-of-the-art technologies to aid you in assessing health hazards and risks of chemical exposures, including the use of tissue culture, omics (e.g., genomics, transcriptomics, proteomics and metabolomics), field studies, computational modelling and artificial intelligence. Additionally you will help to avoid injury from chemicals in both humans and the environment and to manage accidental exposures. You will typically work as part of a multidisciplinary team, which may include other specialists such as computational modelers and histopathologists.

A central theme of research activities carried out at the Toxicology department of Wageningen University is the "3Rs" concept to reduce, refine and replace animal studies 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 requires and has an 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

Career prospects

Employers include universities, private companies in a range of industries, government, regulatory agencies, contract research organizations and consultancies. The tasks you carry out will vary depending on your specific expertise and the job profile, but in general you may need to:

- isolate, identify and measure levels of substances in (food) products and (environmental) samples that may have any harmful effect on humans or the environment
- plan and carry out a range of carefully controlled or field studies on specific chemicals to evaluate whether and how they can be used safely
- help to establish regulations on the use of substances in order to protect public health and the environment
- advise on the safe handling of toxic substances in production or in the event of an accident
- analyze and evaluate statistical data and scientific literature on research outcomes
- write reports and scientific papers, present findings and, in the case of forensic work, give evidence in court

- liaise with regulatory authorities to make sure you are complying with local, national and international regulations and guidelines on chemical use and exposures
- carry out chemical risk assessments
- perform or supervise various tests and trials using specialized techniques, including animal and in vitro toxicity tests
- use experimental data to assess a drug's potential toxicity and identify sensitive individuals
- balance potential benefits against any risks of chemical exposure

Once you have gained experience as a toxicologist, it is possible to move into a senior toxicologist position and then into a management role such as director of toxicology. As your career progresses, you are likely to spend more time managing whole projects, leading teams of staff and overseeing strategy. Becoming a European Registered Toxicologist can enhance your career prospects as it demonstrates your experience and competence in the role.

There are opportunities to progress into project management, having the responsibility of directing others. There are also opportunities to move into consultancy work.

You may find work in the following areas:

academia – obtain a PhD in toxicology, universities or research centers

analytical and clinical laboratories - large district hospitals and specialist regional toxicology units, or contract research organizations

ecotoxicology - environmental hazard assessment in government, water companies, industry and private consultancy

forensic - private forensic laboratories, forensic departments of hospitals or within government departments

industrial and pharmaceutical - various industries including chemical, biotechnology, pharmaceutical, consumer products and food

occupational - within companies or government liaising with the Health and Safety Authorities.

The thesis topics at the Division of Toxicology are divided in 2 major themes:

Human toxicology		Environmental toxicology
Prof Dr Ivonne Rietjens Prof Dr Hans Bouwmeester Dr Nynke Kramer		Prof Dr Nico van den Brink
Ivonne.rietjens@wur.nl Hans.bouwmeester@wur.nl Nynke.kramer@wur.nl		Nico.vandenbrink@wur.nl

For the human toxicology we often study foodborne chemicals.
For arranging an MSc thesis subject you can contact the indicated person (Ivonne, Nico, Nynke or Hans) per theme. For inspiration please see the research topics below categorized by theme.
Please note that apart from the projects given below, 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.

Internships

Finally, after doing a thesis study, there are several opportunities for national or international internships in Toxicology. These internships can be scientific, applied, related to education, policy making, etc. Internship subjects can be arranged for example at WFSR (Wageningen Food Safety Research) or WFBR (Wageningen Food Biobased Research), the Dutch Institute of Public Health and the Environment (RIVM) or elsewhere in the Netherlands or abroad.

This brochure only contain potential MSc thesis topics focussing on human toxicology.

Human toxicology

TOX-H1 Emulate intestinal fibrosis on chip

PhD: Tom Walraven; supervisors: Nynke Kramer, Hans Bouwmeester

The global burden of inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, has been steadily increasing over recent decades, particularly in newly industrialized nations adopting Western lifestyles and diets. More and more studies indicate that widespread intestinal inflammation exacerbates the toxicological effects of drugs, as well as food-borne chemical contaminants. Moreover, the development of intestinal fibrosis, a significant complication observed in IBD, is currently overlooked in chemical and pharmaceutical risk assessment. Despite the urgent need, existing in vitro models fall short in replicating these chronic intestinal diseases effectively. Intestinal fibrosis is characterized as the accumulation fibrous scar tissue in the intestinal wall. The mechanisms of fibrosis are not fully understood, but the overall process is initiated by tissue damage, which is followed by recruitment of inflammatory cells and activation of myofibroblasts, resulting in an overproduction of extracellular matrix (ECM) proteins such as collagen. In the human intestine, ECM is mainly produced by mesenchymal cells of the lamina propria such as fibroblasts. Unfortunately, most current cell culture models for IBD overlook the role of fibroblasts and focus solely on the absorptive cells of the intestine. In this project, we aim to recreate intestinal fibrotic tissues by combining epithelial cells and fibroblasts in one culture system. Stem cells isolated from human tissue form the basis of this project as they are able to give rise to the various cell types found in the intestinal epithelium. These cells can be isolated from healthy and diseased human tissue, and grown as long-term cell cultures in the lab. This innovative approach provides a platform to study the complex interactions between these cells and the exacerbating effects of xenobiotics on intestinal fibrosis.

A thesis project could be: Evaluating how advanced in vitro models of the intestine, such as co-cultures or stem cell cultures, react to fibrotic stressors by examining inflammatory and/or cellular processes.

TOX-H2: The Virtual Human Platform for Safety Assessment

PhD: Thijs Moerenhout; supervisors: Ivonne Rietjens, Hans Bouwmeester, Nynke Kramer

Current legal and regulatory frameworks for the assessment of the safety of chemicals and pharmaceuticals for human health rely predominantly on data from animal studies. However, the accuracy of animal studies to predict toxicity in humans is limited. In addition, current animal testing regimes do not reflect human-relevant scenarios, such as differences in susceptibility due to age, gender, timing of exposure, or disease state. In a national and international arena urgently calling for the reduction of animal testing, the current approach to gradually refine, reduce and replace animal testing has not led to the necessary and desired pace of innovation in animal-free safety assessment. Furthermore, the opportunities offered by state-of-the-art technologies in human health and data science have hardly been yet explored in the realm of safety assessment.

The focus of this project will be on the development of generic PBK models that use a minimum amount of parameters. The generic PBK models are key to ultimately estimate the exposure levels at which adverse health effects in the target organs relevant for the case studies occur, but will essentially also enable inclusion of other target organs. This quantitative in vitro to in vivo extrapolation (QIVIVE) also requires corrections, for protein binding of the compounds of interest under the assay conditions of the in vitro models and the in vivo situation, allowing in vitro exposure data to be translated to in vivo exposure data. In vitro and in silico methods to perform this correction for protein binding will also be studies in more detail. In vitro models suitable to generate the in vitro concentrations response curves will be selected based on so-called adverse outcome pathways (AOPs) taking the molecular initiating event and the mode of action into account.

A thesis project could be: Assessing the passage of human drugs and/or pesticides across a new in vitro blood-brain barrier model, and/or integrating these in vitro derived permeability values in generic PBK models to better predict the disposition and neurotoxicity pesticides in humans. Neurotoxic pesticides to consider include organophosphates, organochlorines, carbamates and rotenone.

TOX-H3: The Virtual Human Platform for Safety Assessment

PhD: Kiri Romano; supervisors: Ivonne Rietjens, Hans Bouwmeester, 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, despite the fact that 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-H4 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 digestable. 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 vido extrapolation (QIVIVE) to assess the dose at which humans will experience these toxicities.

TOX-H5: 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, Ivonne Rietjens

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 neutraceuticals. However, the absoption, 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 a Caco-2 permeability assay and make a structure activity (SAR) model to predict the bioavailability based on the chemical structure of the flavonoid. Techniques to work with include cell culture, analytical chemistry and computational modelling.

TOX-H6: Immunocompetent intestine-on-chip (IoC):

PhD: Germaine Aalderink, supervisors: Hans Bouwmeester, Coen Govers (AFSG-CBI), Tamara Hoppenbrouwers (AFSG-FBR)

The lymphatic vascular system comprises a circulatory network of vessels and lymphoid tissue throughout the body. The lymphatic system is – apart from the circulatory system – the most important transport system in the human body. Lymph nodes and other lymphatic organs filter the lymph to remove microorganisms and other foreign particles. Defense against invading micro-organisms is especially crucial in the small intestine, where the epithelial cells are in direct contact with the luminal microbiota while simultaneously absorbing dietary nutrients. The second function of the lymphatic system in the small intestine is the absorption of fats and fat-soluble compounds from the digestive system and the subsequent transport of these substances to the venous circulation. Lymphatic capillaries in the small intestine contain specialized junctions that allow the uptake of dietary lipids that cannot enter the bloodstream directly. By entering the lymphatic system, lipids and lipophilic compounds circumvent the first-pass metabolism of the liver and distribute across the body. This doctoral project will integrate intestinal epithelial cells, lymphatic endothelial cells, and tissue-resident immune cells within the TissUse™ organ-on-chip platform to study dietary fat absorption and trafficking of immune cells. Moreover, biomimetic micro-scaffolds will be integrated into the organs-on-chip to support intestinal crypt and villi-type organization in the gut. To test functionality, the model will be challenged by microbial-derived compounds and/or metabolites, and effects on barrier integrity, recruitment and infiltration of immune cells, and subsequent inflammatory responses including secretion of inflammatory cytokines.

A thesis project could be: Exploring the culturing of induced pluripotent intestinal cells on Transwells and/or organs-on-chip, developing co-cultures of intestinal and endothelial cells, and challenging these in vitro models with human drugs and or intestinal microbial metabolites.

Another thesis project to assess whether the permeability of the pesticide chlorpyrifos through a Caco-2 permeability assay, which mimics the absorption potential of chlorpyrifos across the gut lining, increases with the addition of fatty foods. This requires the optimization of digestion of lipid matrixes and subsequent extraction of lipophilic compounds from digested matrixes for quantitative measurements.

TOX-H7: Generation of tissue-specific intestinal macrophages and dendritic cells to be included in intestine-on-chip

PhD: Donovan O'Brien; supervisors; Hans Bouwmeester, Coen Govers (ASG-CBI) Tamara Hoppenbrouwer (AFSG-FBR)

Within the intestine, immune cells play and important role in various processes including inflammation, pathogen response and homeostasis. Innate intestinal immune cells more than most other immune cells are unique in that they are consistently exposed to foreign materials, including food antigens, metabolites, pathogenic and commensal bacteria, as well as orally administered compounds resulting in a distinct phenotypic signature epitomized by high tolerance. Two key players within the innate intestinal immune system are macrophages (Mϕs) and dendritic cells (DCs). Broadly, Mϕs represent the 'housekeeping' cell of the submucosal epithelia, phagocytosing apoptotic cells and foreign pathogens alike, as well as modulating secretion to control local inflammation and tissue repair. DCs act as 'sentinel cells', consistently monitoring and sampling the microenvironment, regulating the immune response, and migrating from the site of injury or infection to the lymph node, presenting relevant information to more specialized cells such as T-cells and B-cells.2 Both comprise highly specialized subsets of antigen-presenting cells (APCs) that play a crucial role in linking the innate and the adaptive immune system, and thus are imperative components of any system attempting to model the lymphatic system. Furthermore, with the advancements in recent years in organ-on-chip technologies, the ability to exert greater control as well as biomimetic relevance on systems to model complex organ-specific interactions means that in vitro technologies are now at a point wherein they can be seen as a viable alternative to animal models in accordance with the 3Rs principle.

A thesis project could be: Isolation (of monocytes) and differentiation towards Mϕs and DCs from peripheral blood monocytes or use cell lines like THP-1 cells and expose these cells to tissue-related factors and exogenous compounds which may have an influence on phenotype (e.g. IL-10, retinoic acid, butyrate etc), to derive tissue resident (TR)-like phenotype. This will be assessed by using flow cytometry and fluorescent activated cell sorting (FACS) to analyze phenotype; Using qPCR / RNAseq, determine transcriptional changes in cells and explore functional profile of cells by carrying out cytokine release (ELISA), antigen uptake and phagocytosis assays and inflammatory anergy with TLR stimulation.

TOX-H8: Development of a next generation food safety assessment for mildly processed raw materials.

Post Doc: Frances Widjaja: Hans Bouwmeester, Ivonne Rietjens

The modern food chain may contain natural toxins and anti-nutritional components or undesired microorganisms due to their natural presence in the raw (plant-derived) ingredients or may enter the food chain as contaminants. Subjecting raw materials to mild processing might result in a consumer exposure to natural toxins or specific proteins that are not present (or at very low concentration) in conventionally processed foods or to these unwanted microorganisms. Based on recent literature studies including scientific opinions of the European Food Safety Authority potential chemicals and microbes of concern have been identified. Main organisms will be most probably members of the family Enterobacteriaceae. Interesting groups of chemicals are bacterial toxins, mycotoxins (related to fermentation and mild processing), and natural toxins (alkaloids). Within the group of alkaloids both glycoalkaloids (GAs) including solanines that can be present in potatoes and quinolizidine alkaloids (QAs), that can be present in lupinus, are of interest. In humans, GAs can cause adverse effects including gastrointestinal disturbances such as nausea, vomiting and diarrhoea. In addition, mild processing might affect the fungi and or bacterial contamination. Especially the toxins produced by fungi and bacteria present on the raw materials might be of concern. Selected myco- and bacterial toxins (i.e., DON, aflatoxin, cereulide) will be evaluated in this workstream. Alexandra will develop a next generation risk assessment in which we combine novel toxicological approaches without the use of animal experiments (i.e., in vitro assays) with computational approaches to extrapolate from the observations in vitro to human hazards.

A thesis project could be: Studying the biokinetics of glycoalkaloids, developing PBK models and explore exposure scenario's that include the consequences of differently processed raw materials.

TOX- H9 Assess the biomolecular corona around microplastics and how this affects their uptake of toxicity

Post Doc: Shuo Yang: Hans Bouwmeester, Mathias Busch

Understanding of the relation between physicochemical properties of MNPs and their ability to cross epithelial barriers of the intestinal and lung, is a key requirement to understand the fate of MNPs following human exposure. The oral and inhalation routes of exposure are regarded as the main routes of exposure for MNP. For the intestine, in vitro models using co-cultures of cell-lines based, (or stem cell-based) epithelia will be used that mimic the 3D architecture and cell complexity of the epithelial barriers. To determine and characterise the bio-corona of adsorbed biomolecules (proteins, lipids, sugars, etc.), that forms rapidly on materials upon introduction into a living system we will evaluate how the physicochemical properties of MNPs control the corona composition. For this we will use reversed-phase nanoLC-MS/MS. MNPs will we be incubated in the cell culture media, human lung fluid and intestinal fluid and ideally also from intestinal mucus (in vivo); intestinal chyme (in vitro); (intestinal) microbial preparations. It is highly interesting to study the biocorona of NMPs that have crossed the lung and intestinal barriers and to evaluate the presence of a exogenous molecules in the remaining corona which might trigger immune responses. Particular attention will be given to proteins of the coagulation and complement cascade, which can lead to immunotoxic responses.

A thesis project could be: use of intestinal cell line models (or stem cell derived models) 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-H10 Prenylated flavonoids as novel antimicrobial agents: characterization of their toxicity

PhD: Janniek Ritsema; Supervisors Nynke Kramer (TOX), Carla Araya-Cloutier (FCH)

To combat antimicrobial resistance, novel and effective alternatives to traditional antimicrobial agents need to be developed. Prenylated (iso)flavonoids are potent antimicrobial compounds produced by plants of the Leguminosae family. Prenylation refers to the substitution with an hydrophobic five-carbon isoprenoid unit, and is a structural feature typically associated with increased antimicrobial activity. To effectively apply prenylated (iso)flavonoids as novel alternatives to traditional antimicrobial agents, deeper insights into their mechanism of action and toxicity are required. In this project, which is a collaboration between the laboratory of Food Chemistry (FCH) and Toxicology (TOX), both of these aspects are investigated.

Although prenylated (iso)flavonoids usually have a higher bioactivity than non-prenylated (iso)flavonoids, their relatively low abundance and complex chemical synthesis limits their application in supplements. As a consequence, toxicity data for specifically prenylated (iso)flavonoids is limited. Nowadays, proposing standard animal testing to assess the toxicity of prenylated (iso)flavonoids is ethically problematic and costly given the number of compounds assessed in this project. Indeed, toxicology is moving away from animal testing and towards a more mechanistic approach using new approach methodologies (NAMs), including adverse outcome pathways (AOPs), quantitative structure-activity relationships (QSARs), physiologically based kinetic (PBK) modelling and in vitro toxicity assays. Ideally these NAMs provide insights in the extent of absorption, distribution, metabolism, and excretion in or from the human body (ADME, or toxicokinetics) and the molecular mechanism eliciting a toxic effect resulting in adverse health effects (toxicodynamics). To study the toxicity of prenylated (iso)flavonoids both in vitro and in silico methods will be employed. The cytotoxicity of a structurally diverse set of prenylated (iso)flavonoids will be determined using in vitro cell assays. To gain insights in the structural features of prenylated (iso)flavonoids important for toxicity, QSAR models will be generated using data on the cytotoxicity of prenylated (iso)flavonoids measured in-house and obtained from literature. In addition, the intestinal absorption of prenylated (iso)flavonoids will be studied using the in vitro parallel artificial membrane permeability assay (PAMPA) and Caco-2 permeability assay. This study will increase current understanding of the mechanism of toxicity and structural features of prenylated (iso)flavonoids influencing toxicity.

A thesis topic could be: Assessing the permeability of selected isoflavonoids in the PAMPA and/or Caco-2 permeability assay to assess how prenylation affects the extent to which isoflavonoids are absorbed into the human body through the gut.

TOX-H11 Maintaining concentrations of 'difficult' test chemicals in in vitro toxicity assays constant to improve quantitative in vitro in vivo extrapolations (QIVIVE).

Research assistant: Alexandros Sotiriou; 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-H12: ADME4NGRA: Implementing the EFSA NAMs roadmap through advancing toxicokinetic knowledge in chemical risk assessment

Post Doc: Jingxuan Wang; supervisor: Nynke Kramer

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 advanced in vitro 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- H13 Assess the uptake and toxicity of microplastics

PhD: Tanne Meuwissen; Supervisors: Hans Bouwmeester, Mathias Busch. Note thesis will be preformed at RIVM (Bilthoven or Utrecht)

Nanomaterials (NMs) with photocatalytic ability have successfully been used in disinfection applications for their high effectiveness against bacteria, viruses, and fungi. With an increased interest due to the COVID-19 pandemic multicomponent nanomaterial (MCNM) based antimicrobial coatings to be used on multiple surfaces. To apply a Safe-by-Design approach, the present project will provide data for the human hazard assessment of this next generation of engineered MCNMs by setting up innovative *in vitro* assays. Possible toxicity in the intestine will be investigated by exposing intestinal cell models to these materials (a Caco-2/THP-1 derived macrophage co-culture). Similarly, inhalation exposure of pristine NPs will be assessed using advanced static and dynamic air-liquid interface (ALI) culture and exposure methods for A549 and Calu-3/THP-1 derived macrophage cell models. Thus ultimately might results in the development of, a physiologically based kinetic (PBK) model using *in vitro* systemic exposure data to facilitate Quantitative In Vitro to In Vivo Extrapolation (QIVIVE) for human hazard assessment.

A thesis project could be: Expose in vitro lung models to selected metal-based nanomaterials. For this lung cells will be grown in specific devices that allow an air-liquid exposure. Readouts will be cell toxicity and inflammatory cytokine secretion, amongst other endpoints.

TOX- H14 Development of immunocompetent liver toxicity models to unravel the role of KCs in hepatotoxicity

PhD: Gijs van Slobbe, Supervisors: Coen Govers, 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- H15 Assess the uptake and toxicity of microplastics

Research assistant: Mathias Busch, Supervisors: Hans Bouwmeester

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. Information on the effect of micro and nanoplastics on human health is still preliminary this makes it current very difficult to perform a risk assessment. In this project we use in vitro models of the gastrointestinal barrier to study the effect of gastrointestinal digestion on the uptake and toxicity of the 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-H16: New approach methodologies to assess the role of extra-hepatic metabolism and transport in determining the toxicity of foodborne chemicals during pregnancy

PhD: Chrysanthi Pachoulide; supervisors: Nynke Kramer, Hans Bouwmeester. NB. Supervisor is away 01/09/2024-15/10/2024.

The risk assessment of developmental toxicity predominantly relies on animal studies. However, this approach is subject to limitations not only due to ethical reasons but also due to its inadequacy in accurately predicting toxicity in the dynamic and species-specific conditions of pregnancy and *in utero* development. To simulate the dynamic physiological processes occurring during pregnancy, including placenta formation, maturation and hormonal fluctuations, it is essential to develop and validate New Approach Methodologies (NAMs). Among these methodologies, Physiologically-Based Kinetic (PBK) models are an example, leveraging mathematical simulations to describe the fate(-biokinetics) of a compound in the human body. They predict compound concentrations in specific organs, identify organ-specific accumulation, or bio-activation with minimal input such as organism physiology and compound physicochemical properties. To improve the accuracy of these models, chemical-specific mechanistic kinetic and toxicity data are needed. To address this, mechanistic *in vitro* studies are used to describe processes such as protein binding, metabolic biotransformation and transmembrane transport.

This project aims to develop NAMs relevant for the risk assessment of developmental toxicity of mycotoxins, including enniatins, for which there is currently not enough animal toxicity data to do a traditional risk assessment to assess whether there is a health risk to particularly sensitive individuals like the developing foetus. An understanding of the contribution of gut, liver and placental metabolism and gut and placenta absorption potential as well as the plasma protein binding may be explored using new in vitro models and integrated into PBK models to assess the levels of these mycotoxins reaching the foetus. Embryotoxicity can be determined in vitro too using in vitro stem cell models.

A thesis project could be: Assessing the passage of prototypical drugs and mycotoxins in placental cell model, and/or integrating these in vitro derived data in PBK models to better predict the disposition and toxicity of these chemicals in women.

TOX-H17: Multiomics analysis of advanced glycation end products in organotypic in vitro models

PhD candidate: Haomiao Wang; supervisor: Nynke Kramer, Ivonne Rietjens

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 vitiro and expose these cells in vitro to AGEs wit hand 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-H18: High resolution mass spectrometry methods to help assess the health risk of bee pollen supplements.

PhD candidate: Susannah Heeren; supervisor: Nynke Kramer, Laura Righetti

Food supplements are growing in popularity and are in general considered safe for consumption. Yet, there is no regulation or systematic assessment for the quality and possible health risks of these products. Food supplements often consist of concentrated extracts from natural food products. Given the central tenant in toxicology is “the dose makes the poison”, the lack of regulation may mean that the consumption of supplements might pose a significant health risk. However, the application of traditional toxicity testing and risk assessment approaches for food supplements is too resource intensive and requires many animal tests. Therefore, a new workflow using modern technologies to efficiently assess the health risks associated with supplement consumption is needed. Popular supplements include those that are bee pollen derived. Bee products are well-known for their nutritional and medicinal value. Concurrently they are known be very heterogenous in chemical composition owing to the different geographical origins and lack of regulatory standards for the product. Numerous reports highlight the concerns around possible adverse health effects, occurring with the consumption of these supplements. Bee products are often contaminated with hazardous chemicals such as heavy metals, mycotoxins, pesticides and pyrrolizidine alkaloids. The detection of these pollutants in bee products support the need for a thorough risk assessment. The aim of this PhD project is to develop comprehensive analytical methods to acquire a full picture of the bee pollen composition and simultaneously assess the health risks arising from bee pollen supplements. New approach methodologies in a next generation risk assessment (NGRA) framework will be employed to achieve this aim. Methodologies included are non-targeted analytical methods, quantitative structure activity relationships (QSARs), in vitro cell-based toxicity assays and physiologically based kinetic (PBK) modelling.