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Industrial, biocide and cosmetic chemical inducers of cholestasis

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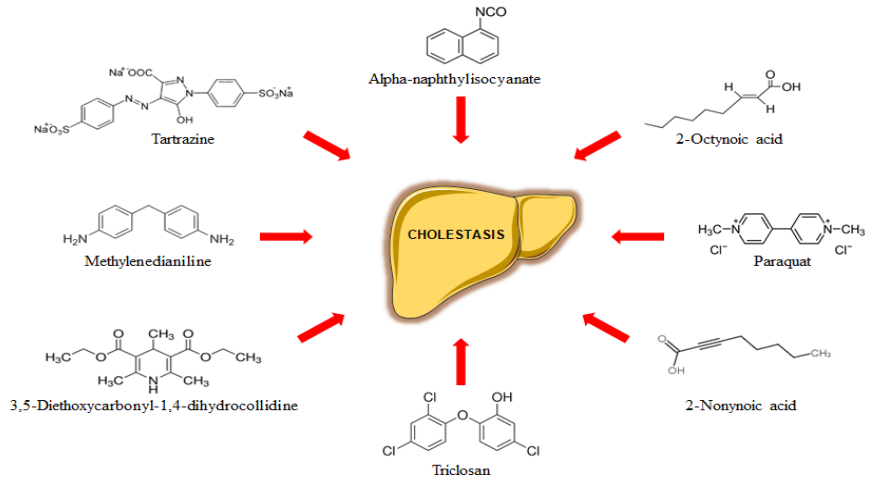
Textual abstract

A frequent side effect of many drugs includes the occurrence of cholestatic liver toxicity. Over the past couple of decades, drug-induced cholestasis has gained considerable attention, resulting in a plethora of data regarding its prevalence and mechanistic basis. Likewise, several food additives and dietary supplements have been reported to cause cholestatic liver insults in the past few years. The induction of cholestatic hepatotoxicity by other types of chemicals, in particular synthetic compounds, such as industrial chemicals, biocides and cosmetic ingredients, has been much less documented. Such information can be found in occasional clinical case reports of accidental intake or suicide attempts as well as in basic and translational study reports on mechanisms or testing of new therapeutics in cholestatic animal models. This paper focuses on such non-pharmaceutical and non-dietary synthetic chemical inducers of cholestatic liver injury, in particular alpha-naphthylisocyanate, 3,5-diethoxycarbonyl-1,4-dihydrocollidine, methylenedianiline, paraquat, tartrazine, triclosan, 2-octynoic acid and 2-nonynoic acid. Most of these cholestatic compounds act by similar mechanisms. This could open perspectives for the prediction of cholestatic potential of chemicals.

Graphical abstract



1. INTRODUCTION

Cholestasis is derived from the Greek words *chole* meaning bile and *stasis* indicating halting, and denotes any situation of impaired bile secretion with concomitant accumulation of bile acids in the liver or in the systemic circulation.[1,2] Depending on the location and cause of the obstruction, a distinction can be made between intrahepatic and extrahepatic cholestasis. Clinically, cholestasis is routinely diagnosed based on biochemical parameters, including increased serum levels of alkaline phosphatase, gamma-glutamyltransferase and bilirubin.[3]

From the mechanistic perspective, cholestatic liver injury can be induced by 3 types of stimuli. First, reduced functionality, expression and/or aberrant subcellular localization of transporters responsible for conveying bile acids and/or drugs may occur, such as the bile salt export pump, multidrug resistance-associated protein 2/3/4 and multidrug resistance protein 3. Second, various hepatocellular changes, including compromised cytoskeletal architecture, disruption of tight junctions and decreased membrane fluidity, may take place. Third, bile canaliculi dynamics may alter. These 3 types of cholestatic triggers induce 2 cellular responses. A first adverse response is elicited by bile acid accumulation and characterized by the occurrence of inflammation, oxidative stress, endoplasmic reticulum stress, mitochondrial impairment and different cell death modes, including necrosis, apoptosis, necroptosis and autophagy. A second adaptive response is aimed at decreasing the uptake and increasing the export of bile acids into and from hepatocytes, respectively, which relies on the activation of nuclear receptors, including the farnesoid X receptor, the pregnane X receptor and the constitutive androstane receptor. These nuclear receptors activate the expression of a number of transporters and enzymes that remove bile acids (Figure 1).[2,4] It should be stressed that the adaptive response is not restricted to the liver, but also takes place in the intestine, kidney and epithelia of the bile duct.[5] In this respect, proliferation of cholangiocytes

leads to corrugations of the luminal duct surface. Accordingly, the surface area increases, duct elongates, branches sprout and loops are formed. Alterations in the bile duct morphology strive to maintain the proximal position of the bile duct relative to the portal vein, which is essential for bile acid transport. Furthermore, this remodeling process enhances resorption of bile acids from the bile duct lumen and transportation to the portal vein.[6,7]

Drug treatment, mainly involving anti-infectious drugs, anti-diabetics, anti-inflammatory drugs, psychotropic drugs, cardiovascular drugs and steroids, frequently underlies the onset of intrahepatic cholestasis.[8,9] As such, drug-induced cholestasis constitutes a subgroup of drug-induced liver injury. The latter is a major reason of drug failure during premarketing and postmarketing phases, accounting for up to 29% of all drug withdrawals.[10,11] In addition to its pharmaceutical relevance, drug-induced liver injury is also of high clinical concern. Indeed, drug-induced liver injury is frequently misdiagnosed, yet it has been estimated to develop in 1 in 100 patients during hospitalization.[12] Furthermore, drug-induced liver injury is responsible for more than 50% of all cases of acute liver failure.[13]

Given its considerable prevalence, drug-induced cholestasis has become well documented throughout the years. This is unlike other types of synthetic chemicals, for which clinical case studies of cholestatic hepatotoxicity are published only sporadically or that are restricted for use in laboratory animals for basic and translational cholestasis research purposes. The present paper gives a concise overview of such atypical synthetic chemical triggers of cholestatic liver injury from the industrial, biocide and/or cosmetic areas.

2. ALPHA-NAPHTHYLISOCYANATE

Alpha-naphthylisocyanate (Figure 2) is used in the preparation of cationic aromatic urethane. It is a model compound to induce intrahepatic cholestasis in laboratory animals, in particular rodents, associated with increased serum levels of alkaline phosphatase and gamma-glutamyltransferase.[14,15]

Metabolomic screening in rat liver following alpha-naphthylisocyanate administration substantiates an overall cholestatic profile, in particular manifested as effects on primary bile acid biosynthesis.[16] Alpha-naphthylisocyanate primarily targets bile duct epithelial cells, but also causes hepatocyte necrosis.[17] In rats, alpha-naphthylisocyanate evokes hepatic inflammation[18], oxidative stress[14], endoplasmic reticulum stress[19] and mitochondrial toxicity[20], all being key events in cholestatic liver injury.[2,4] Alpha-naphthylisocyanate alters the expression and/or activity of a number of hepatic transporters, including the bile salt export pump, multidrug resistance-associated protein 2/3 and multidrug resistance protein 3.[21-23] Furthermore, alpha-naphthylisocyanate disrupts hepatic tight junctions[24,25], decreases membrane fluidity[26] and causes bile canaliculi dilatation[27]. Alpha-naphthylisocyanate decreases levels of glutathione through involvement of phosphoinositide-3-kinase/protein kinase B and nuclear factor erythroid 2-related factor 2 signaling in rat liver, thereby boosting oxidative stress in cholestasis.[28] Alpha-naphthylisocyanate actives the farnesoid X receptor and the pregnane X receptor in mouse liver,[29] which mediate the adaptive response in cholestatic injury.[2-4]

3. 3,5-DIETHOXYCARBONYL-1,4-DIHYDROCOLLIDINE

3,5-diethoxycarbonyl-1,4-dihydrocollidine (Figure 2) is a porphyrinogenic agent and a powerful inducer of delta-aminolevulinate synthetase.[30] It has been used since many decades to

experimentally induce cholestasis in rodents. Thus, administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine to mice increases serum levels of alkaline phosphatase and bilirubin, and triggers histopathological features of inflammation and cell death.[31,32]

Metabolic profiling has confirmed elevated bile acid levels in mice following 3,5-diethoxycarbonyl-1,4-dihydrocollidine treatment.[33] Furthermore, 3,5-diethoxycarbonyl-1,4-dihydrocollidine causes inflammation[34], oxidative stress[35,36], endoplasmic reticulum stress[37], mitochondrial toxicity[35] and apoptosis[38] in mouse liver.

3,5-diethoxycarbonyl-1,4-dihydrocollidine has differential effects on hepatic transporters, including the bile salt export pump and multidrug resistance-associated protein 2[39,40], and suppresses expression of tight junction proteins in liver[40] following administration to mice. In addition, 3,5-diethoxycarbonyl-1,4-dihydrocollidine triggers bile canaliculi dilatation[40] as well as marked derangement of the hepatic cytoskeletal network.[41]

4. METHYLENEDIANILINE

Methylenedianiline (Figure 2) is used in the production of polyamides and epoxy resins as well as in the synthesis of 4,4'-methylenediphenyl diisocyanate, being a major component of polyurethanes. These polymers are applied in the manufacturing of insulation materials, automotive and aircraft parts, and medical devices.[42,43]

Occupational or accidental exposure to methylenedianiline causes injury to bile ducts with subsequent cholestasis, clinically manifested as jaundice, skin rash and elevated serum quantities of alkaline phosphatase and gamma-glutamyltransferase.[44-46] This has been historically termed "Epping jaundice" referring to an accidental mass poisoning in the vicinity of Epping in the United

Kingdom in 1965, during which 84 individuals were poisoned through methylenedianiline-contaminated flour used to make bread.[45,47]

Methylenedianiline diminishes bile flow[48], and causes inflammation, oxidative stress, apoptosis and necrosis in liver upon administration to rats[49] and mice[50]. Methylenedianiline compromises hepatic tight junction integrity and functionality,[51] an effect that only takes place following mitochondrial dysfunction[52]. Recently, a transcriptomic signature of methylenedianiline-induced liver toxicity has been established in rat, thereby confirming cholestasis as a main mechanism of adversity, including effects on the peroxisome proliferator-activated receptor alpha, the liver X receptor, the retinoid X receptor and induction of oxidative stress.[53]

5. PARAQUAT

Paraquat (Figure 2) is a potent herbicide that has been widely used in agriculture as a weed control agent for farmlands and pastures. Accidental or intended ingestion of paraquat results in multiple organ injuries, yet it especially targets the lungs.[54] Nevertheless, paraquat poisoning in humans equally causes cholestatic liver toxicity with extensive jaundice[55] and high serum levels of alkaline phosphatase, gamma-glutamyltranferase and bilirubin[56-61].

Upon administration to mice or rats, paraquat induces hepatic inflammation[62], oxidative stress[63-65] and mitochondrial toxicity[66,67]. This is associated with bile canaliculi dilatation, apoptosis, necrosis and autophagy in liver.[61] Paraquat damages both hepatocytes and bile duct epithelial cells.[68] Paraquat was found to decrease membrane fluidity in mouse liver homogenates.[69] In other cell types, paraquat has shown to disrupt microfilaments[70], to affect liver X receptor activity[71] and to induce Rho-associated protein kinase[72] and c-Jun N-terminal/p38 signaling[71]. Although solid

scientific evidence is currently lacking, these alterations in kinase activity could underlie induction of inflammation and cell death in cholestasis.

6. TRICLOSAN

Triclosan (Figure 2) is a broad-spectrum antimicrobial agent present as an ingredient in several types of personal care products, such as soaps and toothpastes, as well as in detergents, toys, surgical cleaning products and pharmaceuticals.[73]

Triclosan displays antibiotic and antimycotic properties, and acts by interfering with fatty acid synthesis.[74] The latter is not limited to micro-organisms, as triclosan has been shown to induce hallmarks of fatty liver disease in toads[75], frogs[76] and fish[77]. This has been associated with activation of hepatic nuclear receptors, including the pregnane X receptor[78] and the constitutive androstane receptor[79]. These nuclear receptors equally play a key role in cholestasis, in particular by mediating the adverse response and inducing the expression of genes involved in counteracting bile acid accumulation, including those coding for hepatic transporters and biotransformation enzymes.[2,4]

Triclosan causes hepatic inflammation[80], oxidative stress[81], mitochondrial toxicity[82,83], cell cycle arrest and apoptosis[81,84]. Triclosan also suppresses microfilament remodeling and cell membrane ruffling[85], and affects a number of signaling cascades, including protein kinase B[86,87] and extracellular signal-regulated kinases 1/2[87], all that collectively promote cholestatic liver injury. In fact, in oral repeated dose toxicity studies in rodents, triclosan triggers typical diagnostic features of cholestasis, including increased serum levels of alkaline phosphatase, gamma-glutamyltransferase and bilirubin, and induction of liver cell necrosis.[80]

7. TARTRAZINE

Tartrazine (Figure 2) is an orange-colored dye used in cosmetics, textiles, pharmaceuticals and foods.[88] Tartrazine has been linked to primary biliary cholangitis, occurring most frequently in postmenopausal women.[89]

Upon administration to rats or mice, tartrazine causes increases in alkaline phosphatase serum levels, and evokes inflammation, oxidative stress and necrosis in liver.[90-94] Furthermore, tartrazine activates c-Jun N-terminal signaling[95] and mitochondrial toxicity[96] in liver. Tartrazine as well as its sulfonated metabolites act as inhibitors of sulphotransferases.[93,97] Since sulphotransferases play critical roles in sulphatation and hence in secretion of bile acids, inhibition of sulphotransferases is thought to be the main trigger that eventually leads to tartrazine-associated liver toxicity.[93]

8. 2-OCTYNOIC ACID AND 2-NONYNOIC ACID

Primary biliary cholangitis is a chronic progressive cholestatic liver disease accompanied by an anti-mitochondrial antibody response in the vast majority of patients. The auto-antigens recognized by these antibodies are members of 2-oxo-dehydrogenase complexes, particularly the E2 component of pyruvate dehydrogenase. The epitope in the latter includes a lipoyl domain. As a matter of fact, these antibodies also crossreact with a number of chemically modified mimics conjugated to this lipoyl domain,[98,99] among which are 2-octynoic acid[98] and 2-nonynoic acid[99] (Figure 2).

2-Octynoic acid and 2-nonynoic acid are widely used in perfumes, soaps, detergents, lipsticks, toilet waters, facial creams and other perfumed cosmetics because of their violet scent. 2-octynoic acid and 2-nonynoic acid are also applied as food additives, more specifically in flavor compositions for cucumber, berry complexes, fruit blends, peach imitation as well as liqueur

flavorings.[98] Immunization of mice with 2-octynoic acid coupled to albumin evokes auto-immune cholangitis.[100,101] Importantly, sera of patients suffering from primary biliary cholangitis present high antibody reactivity against the E2 component of pyruvate dehydrogenase coupled to 2-octynoic acid, thus underscoring human relevance.[98]

9. VARIOUS SYNTHETIC CHEMICALS

A number of additional chemical compounds, mainly biocides, have been reported, yet less documented, to induce cholestasis. In this regard, several pesticides, including allethrin and tetramethrin, inhibit various human hepatic transporters, some of which play critical roles in bile acid homeostasis.[102,103] The herbicide quizalofop-p-ethyl was found to induce cholestasis in a patient, associated with increased serum levels of alkaline phosphatase, gamma-glutamyltransferase and bilirubin as well as with histopathologically manifested inflammation.[104] A recent study showed that pesticides, such as permethrin and N,N-diethyl-meta-toluamide, aggravate cholestasis in rodents.[105] Yellow phosphorus, an ingredient of certain pesticide pastes and fireworks, is well known to cause hepatotoxicity. In a clinical case study, ingestion of yellow phosphorus was described to increase alkaline phosphatase, gamma-glutamyltransferase and bilirubin serum amounts. Concomitant histopathological examination of the liver revealed intrahepatic cholestasis with inflammation and hepatocyte necrosis.[106]

Nonylphenols are used in manufacturing anti-oxidants, lubricating oil additives, laundry and dish detergents, emulsifiers and solubilizers. They also serve as precursors for the commercially important non-ionic surfactants alkylphenol ethoxylates and nonylphenol ethoxylates, which are used in plastics, pesticides, paints, detergents and personal care products. Polyoxyethylene nonylphenol, as an ingredient of a fungicide product, has been reported to induce irreversible

hepatic injury associated with intracytoplasmic and intracanalicular cholestasis in a patient. Subsequent *in vitro* testing showed the occurrence of necrosis in cultured human hepatocytes exposed to polyoxyethylene nonylphenol.[107]

Diethylhexyl phthalate, also called dioctyl phthalate or bis(2-ethylhexyl)phthalate, is used as a plasticizer in the manufacturing of items made of polyvinylchloride. It is also applied as a hydraulic fluid, as a dielectric fluid in capacitors and as a solvent in glowsticks. Recent evidence suggests cholestatic properties of diethylhexyl phthalate.[108]

Sunset Yellow FCF is a cosmetic, drug and food dye that has been linked to primary biliary cholangitis, occurring most frequently in postmenopausal women.[89] Basic Red 51 is an oxidative and semi-permanent hair dye. In oral repeated dose toxicity studies in rodents, Basic Red 51 increased serum levels of alkaline phosphatase, gamma-glutamyltransferase and bilirubin, and induced liver cell necrosis.[80]

10. CONCLUSIONS AND PERSPECTIVES

Over the past decades, several compounds from a broad chemical space and diverse application areas have been found to induce unexpected and/or unpredicted cholestatic effects. This calls for awareness, not only with risk assessors in governmental agencies and industry, but also in society as a whole. The present paper has reviewed relevant non-pharmaceutical and non-dietary synthetic chemicals that have been described to elicit cholestatic liver insults. For several of these compounds, observations on induction of cholestatic effects directly comes from clinical settings. For other compounds, however, cholestasis-inducing potential has been studied only in laboratory animals and/or in human-based cell cultures, and therefore may need to be verified for actual clinical relevance. Interestingly, most cholestatic compounds act by similar mechanisms,

especially regarding the induction of the deteriorative cholestatic response (Table 1). This could open perspectives for the prediction of cholestatic potential of chemicals of any type and origin. Indeed, current *in vitro* detection of cholestatic compounds is typically based on testing single parameters, such as hepatic transporter inhibition, or on the use of single techniques, like microarrays, yet this has shown to be problematic due to poor predictivity[109] or low sensitivity[11]. The future lies in combining biomarkers and methods that are fully anchored in the mechanistic basis of cholestatic hepatotoxicity. Furthermore, *in vitro* test approaches should be complemented with emerging *in silico* methods that computationally predict cholestatic properties by relying on chemical structure and/or physico-chemical profiles.[2] It can be anticipated that full integration of these methodologies in the upcoming years will enable early and accurate detection of cholestatic chemicals. Such pragmatic strategy can also be of great value for next generation hazard identification of nanomaterials, including nanotitanium oxide, used in household materials, foods and cosmetic products, which might equally evoke cholestatic liver toxicity.[110]

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Author contributions

All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Legends to figures and table

Figure 1

Cholestatic liver injury can be initiated by 3 types of triggering factors, namely (*i*) transporter changes, such as transport inhibition, reduced expression and/or aberrant subcellular localization of bile transporters, (*ii*) hepatocellular changes, including compromised cytoskeletal architecture, disruption of tight junctions and decreased membrane fluidity, and (*iii*) altered bile canaliculi dynamics, namely dilatation or constriction of bile canaliculi. These stimuli induce bile accumulation, which subsequently activates 2 cellular responses, a deteriorative response and an adaptive response. The deteriorative response is typified by the occurrence of mitochondrial impairment, different cell death modes, endoplasmic reticulum (ER) stress with unfolded protein responses (UPR), oxidative stress and inflammation. The adaptive response strives to counteract bile acid accumulation *via* activation of a number of nuclear receptors.

Figure 2

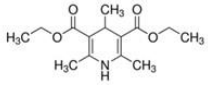
Structure of chemical inducers of cholestatic liver injury.

Table 1

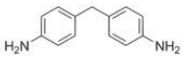
Induction of cholestatic stimuli and responses by industrial, biocide and cosmetic chemicals.

Figure 1

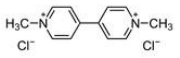
Alpha-naphthylisocyanate



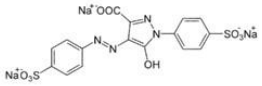
3,5-Diethoxycarbonyl-1,4-dihydrocollidine



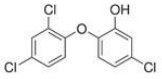
Methylenedianiline



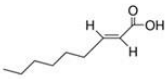
Paraquat



Tartrazine



Triclosan



2-Octynoic acid

2-Nonynoic acid

Figure 2

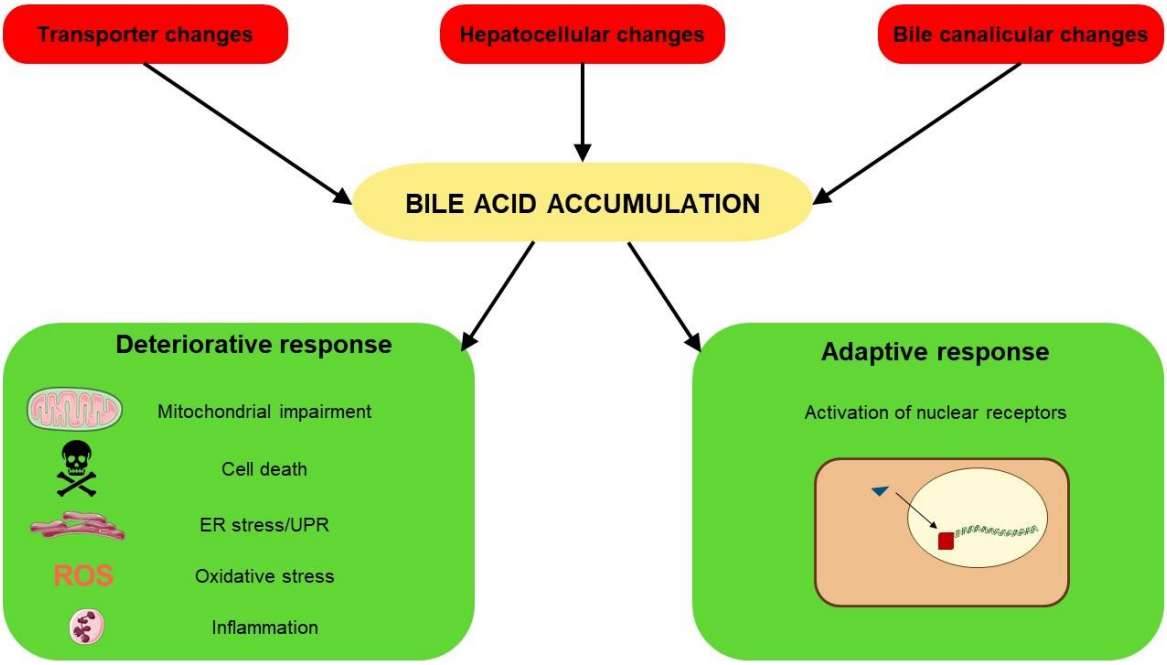

Transporter changes
Hepatocellular changes
Bile canalicular changes
BILE ACID ACCUMULATION
Deteriorative response
Mitochondrial impairment
Cell death
ER stress/UPR
ROS
Oxidative stress
Inflammation
Adaptive response
Activation of nuclear receptors

Table 1

	Effects on transporters	Hepatocellular changes	Altered bile canaliculi dynamics	Inflammation	Oxidative stress	Endoplasmic reticulum stress	Mitochondrial toxicity	Cell death	Nuclear receptor activation
Alpha-naphthylisocyanate	X	X	X	X	X	X	X	X	X
3,5-diethoxycarbonyl-1,4-dihydrocollidine	X	X	X	X	X	X	X	X	
Methylenedianiline		X		X	X		X	X	
Paraquat		X	X	X	X		X	X	
Triclosan		X		X	X		X	X	X
Tartrazine				X	X		X	X	

(references: see text)

Biographies

Vânia Vilas-Boas

Vânia Vilas-Boas graduated as a pharmacist (Pharm.D.) and obtained a doctoral degree in toxicology (Ph.D.) at the University of Porto-Portugal. She is current a postdoctoral researcher at Vrije Universiteit Brussel-Belgium. Her current research interest lies in the application of tridimensional *in vitro* models for therapeutic and (nano)toxicological studies, with specific focus on drug-induced cholestatic injury. She is the author of 18 peer-reviewed publications in international journals.

Eva Gijbels

Eva Gijbels is a doctoral student at Vrije Universiteit Brussel-Belgium. She has a background in pharmaceutical sciences and holds a master degree in drug development (Pharm.D.). Her master thesis was conducted at Columbia University in New York-USA. After her graduation, she started her doctoral thesis project to elucidate the mechanisms of drug-induced cholestasis as the basis for improved animal-free prediction of drug-induced liver injury. She published 2 review papers and 2 book chapters as first author, and co-authored 2 research publications in international peer-reviewed journals.

Axelle Cooreman

Axelle Cooreman is a doctoral student at Vrije Universiteit Brussel-Belgium. She has a background in pharmaceutical sciences and holds a master degree in pharmaceutical care (Pharm.D.). After her graduation, she started her doctoral thesis project to elucidate the role of

connexin and pannexin signaling in cholestasis. She is (co-)author of 4 publications in international peer-reviewed journals.

Raf Van Campenhout

Raf Van Campenhout is a doctoral student at Vrije Universiteit Brussel-Belgium. He has a background in pharmaceutical sciences and holds a master degree in drug development (Pharm.D.). After his graduation, he started his doctoral thesis project, which is focused on the production of novel inhibitors of pannexin signaling. He is co-author of 4 publications in international peer-reviewed journals.

Emma Gustafson

Emma Gustafson graduated as a master in medical science with major in toxicology at the Karolinska Institutet-Sweden. She conduced her master thesis in the area of translational nanomedicine at Trinity College-Ireland. She currently is a doctoral student at Vrije Universiteit Brussel-Belgium. Her project aims to test the applicability of a generic strategy using *in vitro* and *in silico* tools for animal-free risk evaluation of chemicals with a focus on hepatotoxicity in a repeated exposure scenario. She co-authored 2 publications in international peer-reviewed journals.

Kaat Leroy

Kaat Leroy graduated as a master of science in biomedical sciences at Universiteit Gent-Belgium and currently is a doctoral student at Vrije Universiteit Brussel-Belgium. Her doctoral thesis project specifically addresses the role of connexin and pannexin signaling in liver cancer. This

project aims to identify new biomarkers, drug targets and therapeutics for a better prognosis and treatment of liver cancer.

Mathieu Vinken

Mathieu Vinken is an associate professor affiliated to the Vrije Universiteit Brussel-Belgium. He has a background in pharmaceutical sciences (Pharm.D.), holds a doctoral degree in experimental *in vitro* toxicology (Ph.D.) and is a European Registered Toxicologist (E.R.T.). He is President of the European Society of Toxicology *In Vitro*. He is author of more than 150 publications in international peer-reviewed journals and books. He is editor of 3 books. He is associate editor of the journals Toxicology *In Vitro* and Archives of Toxicology as well as European editor of the journal Applied *In Vitro* Toxicology.