The liver is the major organ involved in the metabolism and excretion of the majority of drugs and toxins that are introduced into the body. Parent drugs or their metabolites can cause hepatotoxicity, that lead to drug-induced liver injury (DILI). DILI has an estimated annual incidence between 10 and 15 per 10,000 to 100,000 persons exposed to prescription medications [1, 2, 3, 4]. DILI is also the most frequently cited reason for withdrawal of medications from the market [5, 6]. DILI may not be detected in the pre-clinical and clinical studies prior to drug approval, because most new drugs are tested in fewer than 10,000 people prior to drug approval. As a result, DILI with an incidence of 1 in 10,000 may be missed. Apart from the safety issues, drug development is a time consuming process (10-15 years) and huge costs ($2.6 billion) are involved before a new drug is approved on the market [7]. As a consequence, intensive efforts are being made both in academia and industry to develop biomarkers and methodologies to assess hepatotoxic effects as early as possible. The methods include quantitative structure activity relationship assessments, in vitro assays, high-content screening assays and omics studies [8, 9]. Currently, hepatotoxicity is evaluated in in vivo repeated-dose toxicity tests in animals by analysis of hematological, histopathological and clinical parameters. However, these parameters can generate false negative results due to their insensitivity [10, 11] or due to species differences. In addition the predictive value of these preclinical studies is limited [12]. This emphasizes the need for novel screening methods that facilitate the early assessment of the toxic potential of new molecules []. These new screening methods are preferably applied on in vitro test systems to reduce the number of laboratory animals. In addition preferably human derived systems should be used to avoid interspecies extrapolation. To improve the sensitivity of the preclinical parameters, omics-technologies have been developed and in particular the transcriptomics based screenings have already shown promising results for improving the current toxicity tests [8].

The development of predictive models is also hampered by the wide variety of phenotypes of liver injury. DILI is mainly classified into intrinsic (dose-dependent, reproducible) and idiosyncratic (low incidence and largely dose-independent) types. Intrinsic DILI results in different phenotypes of toxic injury based on acute or chronic exposure to drugs such as apoptosis, necrosis, cholestasis, steatosis, fibrosis or cirrhosis. Whereas idiosyncratic drug-induced liver injury (IDILI) is a rare adverse drug reaction of which the mechanism is still poorly understood and which can lead to cholestatic or hepatocellular injury resulting in liver failure, or even death.

1.1 LIVER TOXICITY PHENOTYPES

In this thesis, we focused on four toxic phenotypes: cholestasis, fibrosis, necrosis and idiosyncratic toxicity. Some mechanistic details underlying the studied toxic phenotypes are outlined here.
**Liver Necrosis**  Necrosis is caused by acute metabolic perturbation that leads to ATP depletion. Drug-induced cell necrosis results from an intense and massive perturbation of cell homeostasis, with ATP depletion associated with cytoskeletal alterations, cellular swelling and bleb formation and rupture of the lysosomal membrane resulting in release of lysosomal enzymes and irreversible collapse of electrical and ion gradients [13]. The clinical course of acute hepatic necrosis resembles an acute, toxic injury to the liver with sudden and precipitous onset, marked elevations in serum aminotransferase levels, and early signs of hepatic (or other organ) dysfunction or failure despite minimal or no jaundice. Rapid recovery after withdrawal of the causing agent is also typical. Acute hepatic necrosis is typically caused by a direct hepatotoxin and is usually dose dependent and intrinsic, rather than idiosyncratic. In many cases a reactive metabolite is involved that covalently binds to tissue macromolecules.

**Liver Cholestasis**  Cholestasis is a condition characterized by inhibition of bile flow from the liver to the bile ducts, which may damage the liver. Cholestasis accounts for 40-50% of all reported DILI cases [14, 15]. The main causative event involved in drug-induced cholestasis is assumed to be BSEP (Bile Salt Export Pump) inhibition by drugs. As a result of this, toxic bile acids accumulate in the hepatocytes or bile canaliculi [16, 17, 18]. These bile salts trigger an adaptive response and a direct deteriorative response. Adaptive response activation counteracts bile accumulation and thus cholestatic liver injury. A complex machinery of transcriptionally coordinated mechanisms mediated by FXR, LXR, CXR and PAR nuclear receptors is activated by bile acids, which collectively decrease the uptake and increase the export of bile acids into and from hepatocytes, respectively. Also, detoxification of bile acids is enhanced, while their synthesis becomes downregulated [19, 16, 17, 18]. Despite the activation of these protective pathways, a deteriorative response occurs, accompanied by mitochondrial impairment, inflammation, the production of reactive oxygen species and ultimately to the onset of cell death by both apoptotic and necrotic mechanisms. Recently, Vinken et al proposed an Adverse Outcome Pathway for cholestasis (fig. 1), which describes the mechanism of cholestasis from the first molecular interaction between the toxin and the cell, via the cellular effects to the effect on the tissue and the final outcome for the organism [16, 17, 18]. Proposed key events are the accumulation of bile acids, the induction of oxidative stress and inflammation, and the activation of nuclear receptors.

**Liver Fibrosis**  Liver fibrosis is the scarring process that represents the liver’s response to chronic cellular injury and reflects an imbalance between liver repair and scar formation [20]. A central event in liver fibrosis is the activation of hepatic stellate cells to adopt a myofibroblasts like phenotype [21]. Different key events at the cellular and tissue level include hepatocyte injury and cell death, activation of Kupffer cells, expression of transforming growth factor beta 1, activation of hepatic stellate cells, oxidative stress and chronic inflammation, collagen accumulation and changes in hepatic extracellular matrix composition [22]. CCl₄ intoxication, which is a well-known inducer of fibrosis in rat and mouse, is most studied as a model to understand mechanistic events in liver fibrosis [23]. Also for fibrosis an adverse outcome pathway was proposed (fig. 2), clearly indicating the involvement of several liver cell types [22].
1.1 Liver Toxicity Phenotypes

**Figure 1:** Adverse outcome pathway for cholestasis (reprinted with permission from [18])

**Figure 2:** Adverse outcome pathway for fibrosis (reprinted with permission from [22])
Idiosyncratic DILI  Idiosyncratic drug-induced liver injury differs from intrinsic toxicity in that IDILI is not directly reproducible in animal models; not strictly dose-dependent (although it occurs mainly with drugs that are dosed at a relatively high dose); variable and often delayed time of onset, variable liver pathology and usually not related to the drug’s pharmacologic mechanism of action. To illustrate the latter, clozapine is associated with IDILI whereas olanzapine is not. Although the risk of acute liver failure associated with idiosyncratic hepatotoxins is low (about 1 in ten thousand patients) there are more than 1,000 drugs and herbal products associated with this type of toxic reaction [24, 25].

Several hypothesis are being tested to understand the mechanisms of IDILI drug reactions such as the inflammatory stress hypothesis, hapten hypothesis, failure to adapt hypothesis, danger signal hypothesis, reactive intermediate hypothesis and mitochondrial stress hypothesis (fig. 3) [25, 26, 27]. The inflammatory stress hypothesis is based on the assumption that an acute episode of inflammation has the potential to interact with the concurrent drug therapy to precipitate IDILI. Inflammatory stress models in rodents have suggested the potential role for inflammatory stress in the mechanism of human IDILI. Substantial evidence for interactions between IDILI-causing drugs and inflammation has been reported in these rodent models, which suggests that inflammation plays a role in the idiosyncratic toxicities induced by some well-known IDILI drugs [26]. However, it is also likely that various other mechanisms (fig. 3) alone or in combination, are involved in idiosyncratic toxicities.
1.2 in vitro MODEL SYSTEMS TO STUDY DILI

In vivo animal studies are the toxicological gold standard for the assessment of the toxic effect of chemicals. However, this type of study is time consuming, expensive and causes suffering of the animals. Another problem is the increasing number of compounds that have to be tested (among others as a result of the REACH initiative), making the in vivo studies not eligible. Although animal studies are an important and useful tool and have to be performed due to guidelines, there are limitations, and a study by Olson et al. showed that half of the drugs that are hepatotoxic in humans did not have the same effect in animals. This study included 221 drugs and the concordance for liver toxicity in humans and experimental animals was 55%, which is only slightly better than tossing a coin, and which is much lower than for other organs, such as the gastrointestinal (85%) and cardiovascular (80%) system [28, 29, 30]. Moreover, in order to reduce the number of animals used in these studies and to increase the possibilities for detailed mechanistic studies, in vitro models were developed to evaluate the safety of compounds. In vitro models in general are more cost effective and contribute to the replacement, reduction, and refinement of animal testing [31]. For the hepatic metabolism and toxicity evaluation of drugs several in vitro models are currently used: the isolated perfused liver (i.e. the intact organ), liver slices, isolated cell preparations (e.g. hepatocytes, or cocultures of hepatocytes with other cell types), cell lines (e.g. HepaRG, HepG2), subcellular fractions (e.g. microsomes), expressed enzymes and in silico models [32]. Stem cell derived hepatocytes are currently under development, which will enable ample availability of differentiated human cells [33].

Drug-induced liver injuries are caused by complex processes, which involve numerous cell types and mediators, making toxicity studies in isolated hepatocytes or liver cell lines incomplete. In contrast, the ex vivo model of precision-cut organ slices retains the same multicellular, structural and functional characteristics as the in vivo tissue. This model allows a better understanding of cell interactions involved in drug-induced injuries. Precision-cut liver slices (PCLS) retain the organ architecture and compared to primary hepatocytes, they allow the analysis of regional toxicity (e.g. zonal effects across the liver lobule) and the study of the role of all liver cell types in hepatic toxicity [34]. Since the tissue architecture is maintained, the effects of toxicants can be evaluated with morphological techniques (e.g. histological evaluation, immunocytochemistry) in addition to the clinical biochemistry tests [32]. This model can be used for different species and organs (e.g. liver, kidney, lung, brain, small intestine and colon), allowing cross-species and inter-organ comparisons [34]. Although this model has been successfully applied to human liver tissue, like for all in vitro models prepared from human tissue, the availability of human tissues limits the throughput of human PCLS. Advantages and disadvantages of the PCLS model in comparison to other in vitro systems are summarized in table 1.
Table 1: Summary of commonly used *in vitro* hepatotoxicity model systems

<table>
<thead>
<tr>
<th>System</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Liver slices</td>
<td>- retained organ complexity, cell polarity, intercellular and cell-matrix contacts</td>
<td>- exposure and activity of cells in slices can vary</td>
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<td></td>
<td>- similar preparation for all species</td>
<td>- lifespan limited to 5 days</td>
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<td></td>
<td>- several organs from the same donor can be studied and cocultured</td>
<td>- no cryopreservation available yet</td>
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<tr>
<td></td>
<td>- histological and biochemical tests</td>
<td>- low throughput</td>
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<tr>
<td></td>
<td>- intra-organ regional differences</td>
<td>- development of fibrosis in cultures longer than 2 days</td>
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<td></td>
<td>- functional bile canaliculi</td>
<td></td>
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<tr>
<td>Primary hepatocyte suspensions</td>
<td>- short time maintenance of <em>in vivo</em> function</td>
<td><em>in vitro</em> lifespan &lt; 3 hours</td>
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<tr>
<td></td>
<td>- cryopreserved hepatocytes available</td>
<td>- rapid and progressive loss of <em>in vivo</em> properties</td>
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<tr>
<td></td>
<td>- medium-high throughput</td>
<td>- loss of cell polarity</td>
</tr>
<tr>
<td>Hepatocyte monocultures</td>
<td>- polarity partly restored in sandwich cultures</td>
<td>- limited interactions between cells</td>
</tr>
<tr>
<td></td>
<td>- cryopreserved hepatocytes available</td>
<td>- absence of other cell types</td>
</tr>
<tr>
<td></td>
<td>- medium-high throughput</td>
<td>- loss of differentiation and drug metabolism</td>
</tr>
<tr>
<td>Co-cultures of hepatocytes and other cells</td>
<td>- better maintenance of differentiation</td>
<td>- conflicting cell culture requirements for different cells</td>
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<tr>
<td></td>
<td>- cryopreserved cells available</td>
<td>- non-physiological orientation</td>
</tr>
<tr>
<td></td>
<td>- specific cell-cell interactions can be studied</td>
<td>- complex procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- low throughput</td>
</tr>
<tr>
<td>Cell lines</td>
<td>- readily available</td>
<td>- loss of differentiation</td>
</tr>
<tr>
<td></td>
<td>- relatively reproducible</td>
<td>- cancer cell properties</td>
</tr>
<tr>
<td></td>
<td>- easy preparation</td>
<td>- lacking many functional characteristics of liver tissue</td>
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<tr>
<td></td>
<td>- cryopreserved cell lines available</td>
<td></td>
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<tr>
<td></td>
<td>- restored polarity in some cell lines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- high throughput</td>
<td></td>
</tr>
<tr>
<td>Stem cell-derived hepatocytes</td>
<td>- once successfully differentiated, ample availability</td>
<td>- full differentiation not yet achieved</td>
</tr>
<tr>
<td></td>
<td>- high throughput</td>
<td>- complex preparation</td>
</tr>
<tr>
<td></td>
<td>- patient-specific cells</td>
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<tr>
<td></td>
<td>- cryopreservation possible</td>
<td></td>
</tr>
<tr>
<td>Organoid cultures</td>
<td>- restored cell contacts and polarity</td>
<td>- technically complex</td>
</tr>
<tr>
<td></td>
<td>- patient-specific cells</td>
<td>- low-medium throughput</td>
</tr>
<tr>
<td></td>
<td>- cryopreservation possible</td>
<td>- loss of differentiation</td>
</tr>
<tr>
<td>Perfused liver</td>
<td>- biliary excretion functions intact</td>
<td><em>short life span</em> (&lt;3h)</td>
</tr>
<tr>
<td></td>
<td>- maintenance of organ complexity, intercellular contacts and cell polarity</td>
<td>- difficult to apply to human livers</td>
</tr>
<tr>
<td></td>
<td>- intact blood/medium supply through the sinusoids</td>
<td>- very low throughput</td>
</tr>
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</table>
MODEL COMPOUNDS

For the research studies described in this thesis, prototypical hepatotoxic compounds were selected based on extensive literature search, that are known to induce a specific phenotype of toxicity such as necrosis, cholestasis, fibrosis and IDILI, based on their known in vivo toxicity. These well-defined hepatotoxicants served to elucidate the mechanisms underlying the toxic phenotype and classify the hepatotoxicants based on their phenotype of toxicity. The compounds studied in this thesis are briefly described here.

Necrotic compounds

Hepatotoxicants that were described to induce liver necrosis only, with limited or no signs of cholestasis, were selected by literature search and also by using the histopathology data from the TG-GATEs toxicogenomics database (http://toxico.nibiohn.go.jp/).

ACETAMINOPHEN  Acetaminophen (paracetamol, APAP) is a widely used over-the-counter drug, used for its analgesic and antipyretic effects. At therapeutic doses, APAP is considered a safe drug, but at higher doses it can produce centrilobular hepatic necrosis, that can be fatal to the patient. APAP hepatotoxicity is by far the most common cause of acute liver failure [35]. The liver injury is attributed to its reactive metabolite (NAPQI), which among others shows mitochondrial toxicity.

BENZIODARONE  Benziodarone (BZ) is a vasodilator and a uricosuric agent. It was withdrawn in 1964 due to hepatotoxicity [36]. The compound has a chemical structure similar to benzbromarone, a well-known hepatotoxic agent [37].

CHLORAMPHENICOL  Chloramphenicol (CH) is a broad-spectrum antibiotic extracted from the bacterium Streptomyces venezuelae. An in vivo study in rats showed that CH has necrotic effects on the liver [38]. In humans, acute liver necrosis was reported after CH therapy [39]. Oxidative stress is possibly involved in the CH induced hepatotoxicity [40]. One isolated case of cholestatic jaundice was reported [41].

COLCHICINE  Colchicine (CL) is a natural product and secondary metabolite, originally extracted from plants of the genus Colchicum used in treatment of gout. Colchicine is known to cause hepatic necrosis and inhibition of microtubule or spindle formation and mitotic arrest were suggested to be the mechanisms of colchicine hepatotoxicity [42, 43].

N-NITROSODIETHYLAMINE  N-nitrosodiethylamine is a carcinogen, and it is used to induce hepatocellular carcinoma in animal experiments [44]. It is also shown to induce hepatic necrosis in mice in vivo [45]. Generation of reactive oxygen species (ROS) that results in oxidative stress or cellular injury is the suggested mechanism involved in hepatotoxicity [45].

Cholestatic compounds

Hepatotoxicants, which are known to induce cholestasis by different mechanisms, were chosen from the literature.
1-NAPHTYL ISOTHIOCYANATE (ANIT) ANIT is a model compound which causes cholestasis in experimental animals [46]. ANIT induced cholestasis involves direct injury to bile ducts, called cholangiodestructive cholestasis [47].

CYCLOSPORINE (CS) Cyclosporine is an immunosuppressant drug widely used in organ transplantation to prevent organ rejection. Cyclosporine is known to induce cholestasis in kidney, heart and liver transplant patients [48, 49]. CS is a potent inhibitor of BSEP and BSEP inhibition is the mechanism involved in the CS induced cholestasis [47, 50].

CHLORPROMAZINE (CP) Chlorpromazine is a antipsychotic drug being used to treat schizophrenia. Chlorpromazine treatment was observed to cause hepatocanalicular jaundice in 1% of patients within 1-5 weeks of treatment. Chlorpromazine-induced cholestasis is associated with hypersensitivity or idiosyncratic reaction resulting in cholestatic hepatitis [47]. Other mechanisms have also been suggested, including inhibition of bile flow, inhibition of Na+-K+-ATPase function and an alteration of membrane fluidity.

ETHINYL ESTRADIOL (EE) Ethinyl estradiol is an orally active estrogen used in many formulations of oral contraceptive pills. Pure cholestasis without hepatitis characterized by selective interference with bile excretory mechanisms is observed with ethinyl estradiol [47]. Interference of the glucuronide metabolite of EE with bile excretory transporters (BSEP) is involved in the ET induced cholestasis.

METHYL TESTOSTERONE (MT) Methyl testosterone is used as anabolic steroid. Cholestatic jaundice is observed in a patient with normal or mild elevation of alkaline phosphatase [51]. As like EE, interference with bile excretory mechanisms is involved in the MT induced cholestasis. Pure cholestasis without hepatitis is also observed with methyl testosterone in rats [47, 52].

Idiosyncratic compound

In order to apply toxicogenomics to study the possible mechanisms involved in IDILI, clozapine, which is known to cause drug-induced IDILI in humans was considered.

CLOZAPINE Clozapine is an atypical antipsychotic medication used in the treatment of schizophrenia. Idiosyncratic reactions associated with clozapine include cholestatic liver injury as evidenced by an increase in serum \( \gamma \)-glutamyl transferase (GGT) activity in humans [53]. A non-injurious dose of LPS and nontoxic dose of clozapine in rats resulted in significant increases in serum liver enzymes which did not occur with neither clozapine nor LPS alone [54]. As a non-idiosyncratic pharmacological analogue to clozapine, olanzapine was included in the studies to be able to separate the pharmacological effects from the IDILI. Olanzapine is also an atypical antipsychotic drug and is considered a safe alternative in the treatment of refractory schizophrenia [55].
1.3 Toxicogenomics

Drug induced liver injury is traditionally assessed in preclinical studies using clinical biomarkers. Clinical biomarkers such as alkaline phosphatase, alanine amino-transferase, asparate amino-transferase, gamma glutamyl transferase and bilirubin can give valuable information about liver injury due to drugs but their lack of sensitivity and specificity challenges the identification and differentiation of different liver toxic phenotypes. The current biomarkers for liver injury are reflecting the amplitude of the organ damage rather than the mechanism of action of toxicants. Also, these enzymatic or endogenous markers are released in blood (or medium) as a result of irreversible damage of the cell membrane due to necrosis and mostly reflect a late response to the drug-induced injury. Drug-induced liver injury is the result of alteration of biological processes induced by a drug or its metabolite, resulting in toxic effects. Toxicogenomics deals with the collection and interpretation of information about gene, metabolite and protein expression within a particular cell or tissue in response to toxic substances [56]. It serves to elucidate the molecular mechanisms involved in the toxicity, and to derive molecular expression patterns (i.e. molecular biomarkers) that can predict toxicity [57, 58, 59, 8, 60]. Toxicogenomics combines toxicology with genomics, and uses high throughput molecular profiling technologies such as transcriptomics, proteomics and metabolomics and is being used in the academia and industry for more than a decade to study toxic effects of pharmaceutical drugs or environmental chemicals in various ex vivo, in vitro and in vivo model systems in order to predict the risk to patients or the environment [11, 60].

Transcriptomics

Transcriptomics is the study of the transcriptome (the complete set of RNA transcripts) that is produced by the genome, under specific conditions using microarray analysis. Comparison of transcriptomes allows the identification of differentially expressed genes in response to different drug treatments. Toxic drugs can cause alterations in the expression of genes, leading to the interruption of the corresponding biological functions, networks and pathways that are of importance for the normal functioning of the organ [61]. Hence, the alterations in the levels of expression of these genes can reflect underlying toxicity mechanisms [62]. There is also evidence that suggests that the gene expression changes in the target organs present before the appearance of the classical biochemical and histological indicators of toxicity [63, 64], thereby elucidating early events. As such, the determination of changes in gene expression of selective gene markers in the target organs in response to the exposure to toxic drugs helps in the pre-clinical diagnosis of toxic endpoints and in turn helps in the selection of drug candidates. Moreover it may be helpful to design effective intervention strategies for preventing adverse effects. The gene expression data provides insights in the possible mechanisms underlying the toxicity and may also be used to identify biomarkers of early toxicity.
1.4 AIM AND SCOPE OF THE THESIS

The research described in this thesis was focused on the use of precision-cut liver slice (PCLS) as an *ex vivo* model in combination with transcriptomics analysis to predict and understand the possible mechanisms of intrinsic and idiosyncratic DILI. The ultimate goal of this research is to contribute to a better early identification of drugs that cause intrinsic or idiosyncratic toxicity in humans before the drug forward to further preclinical and clinical evaluation, with concurrent reduction in the use of experimental animals.

In Chapter 2, rat PCLS was validated as an *ex vivo* model to identify the fibrotic potential of toxic compounds after short-term exposure using a transcriptomics approach. In rat *in vivo*, both paracetamol (APAP) and carbon tetrachloride (CCl₄) induce liver necrosis, but long-term treatment with CCl₄, in contrast to paracetamol, causes liver fibrosis. The aim of this study was to perform transcriptomic analysis to compare the early changes in mRNA expression profiles induced by APAP and CCl₄ in the rat PCLS and to identify early markers that could predict fibrosis-inducing potential.

In Chapter 3, the human PCLS model was validated as an *ex vivo* model to reflect drug-induced cholestasis and to identify the possible mechanisms of cholestasis-induced toxicity using gene expression profiles. Five hepatotoxicants, which are known to induce cholestasis (alpha-naphthyl isothiocyanate, chlorpromazine, cyclosporine, ethinyl estradiol and methyl testosterone) were tested in the presence of a non-toxic concentration of a physiological bile acid mixture. This non-toxic bile acid mixture (60 µM) was added to the incubation medium in order to create an environment similar to that in the portal vein of human *in vivo*. We aimed to verify whether human PCLS incubated with these cholestatic drugs in the presence of this physiological bile acid mixture, correctly reflect the pathways affected in drug-induced cholestasis in the human liver.

In Chapter 4, we aimed to confirm the hypothesis that hepatotoxicants can be classified according to their phenotype of toxicity using human PCLS *ex vivo*, we tried to classify known hepatotoxicants on their phenotype of toxicity using gene expression profiles. Hepatotoxicants that are known to induce either necrosis (n=5) or cholestasis (n=5) were tested at concentrations inducing low (<30%) and medium (30-50%) toxicity. Random forest (RF) and support vector machine (SVM) algorithms were used to identify classifier genes, which can discriminate hepatotoxicants based on the phenotype of toxicity.

Apart from testing PCLS as a suitable model to study drug induced intrinsic toxic phenotypes such as fibrosis, cholestasis or necrosis, the PCLS model was also used to study drug induced idiosyncratic toxicity. Recently, Hadi et al successfully developed and validated the human PCLS as a model, based on the inflammatory test hypothesis, to discern IDILI-related drugs from non-IDILI-related drugs, using human PCLS co-incubated with IDILI drugs and lipopolysaccharide [65].

In Chapter 5, we carried out a transcriptomic analysis to identify possible biomarkers and pathways responsible for IDILI using clozapine as a well-known IDILI drug and olanzapine as its non-IDILI-associated analogue.

Finally, Chapter 6 presents a summary and general discussion of the major findings of all studies presented in this thesis and a discussion of the future perspectives of the use of human PCLS as model for the research on drug-induced toxicity in man.