Chapter 2

Aim and Outline of the thesis
The intestine is an important target for drug-induced toxicity due to its high exposure after oral administration. The prediction of drug induced intestinal side effects remains a significant safety issue in pharmaceutical development. *In vivo* experiments to study intestinal toxicity induced by chemicals in human are difficult to perform due to ethical constraints. Animal experiments have been proven useful, however, species differences and the requirement for reduction of animal use warrant the development of *in vitro* methods that can also be applied to human tissue. Currently a few *in vitro* methods are available to study intestinal toxicity. However, low viability and lack of metabolism capacity remain the common shortcomings. Moreover they do not reflect the cellular heterogeneity of the intestine nor the gradients along the length of the organ. Therefore an *in vitro or ex vivo* method for drug-induced toxicity that reflects the organs characteristics more accurately and that can be applied to human tissue is highly desired.

**The aim** of the research described in this thesis was to investigate the applicability of precision-cut intestinal slices to study drug-induced toxicity in the intestine *ex vivo*.

In Chapter 1, the precision cut intestinal slice (PCIS) technology is introduced as a new addition to the battery of *in vitro* assays for evaluation of xenobiotic toxicity, metabolism and transport. PCIS can be prepared from each region of the intestine and from various species in a similar manner. They contain all the intestinal cell types in their natural matrix and maintain high activities of enzymes and transporters involved in drug disposition during culture up to 8-24 hours. Since drug-induced toxicity is usually a result of interaction between multiple cell types, and drug metabolism and transport plays a critical role in toxicity, PCIS are a potentially promising model to study drug-induced toxicity *ex vivo*, and offer the opportunity to apply it to human tissue.

Chapter 3-5 of this thesis focus on the mechanism of non-steroidal anti-inflammatory drugs-induced intestinal toxicity using PCIS as an *ex vivo* model from rat and human intestines. Non-steroidal anti-inflammatory drugs (NSAIDs), commonly administered for rheumatic and arthritic diseases, are notorious for their high prevalence of side effects in the small intestine including bleeding, ulceration, inflammation or perforation in humans and experimental animals, especially rodents. Multiple mechanisms have been reported to contribute to their enteropathy, and these mechanisms are shared by most of the NSAIDs. In Chapter 3, firstly, one of the most extensively studied NSAIDs, diclofenac (DCF), was used as a model compound to test the rat PCIS system. In addition to a concentration-effect relationship, it was investigated to what extent the mechanisms of the DCF toxicity described in the literature were reflected in the PCIS using biomarkers for electrophile stress (induced by reactive metabolites), endoplasmic reticulum stress, mitochondrial injury, and oxidative stress. Secondly, rat PCIS were incubated with a concentration range of five NSAIDs (diflunisal, diclofenac, indomethacin, naproxen, aspirin) to test whether PCIS could predict
their toxicity. The *ex vivo* toxicity ranking obtained from the TC50 values was compared with published *in vitro* and *in vivo* data.

After proving in **Chapter 3** that rat PCIS can correctly reflect the multiple mechanism of DCF-induced intestinal toxicity, DCF toxicity was further explored in human PCIS. In **Chapter 4**, PCIS prepared from the jejunum of 18 human donors were used, and DCF-induced direct toxicity to human PCIS, was studied by ATP depletion, morphological damage, caspase 3 activation and LDH leakage. Subsequently, the intestinal metabolism of DCF and resulting protein adduct formation were thoroughly studied at different time points, at different DCF concentrations, and in the presence or absence of inhibitors of DCF metabolizing enzymes.

One of the advantages of using an *ex vivo* model to study the intestinal toxicity is that the influence of the liver (especially the liver metabolites) can be excluded. It has been reported that Mrp2 deficient rats were resistant to DCF induced intestinal toxicity due to the impaired Mrp2-mediated transport of liver DCF metabolites to the intestine. However, Mrp2 is also located in the intestine, and it is not clear whether adaptive changes induced by the Mrp2 deficiency in the intestine, especially the changes in DCF disposition, will also contribute to the reduced sensitivity. In **Chapter 5**, the consequences of Mrp2 deficiency including transporter gene expression, GSH content, DCF metabolism, disposition and intrinsic toxicity were compared between Mrp2 deficient rat intestine and their wild type counterparts by two *ex vivo* setups: PCIS and Ussing Chamber.

PCIS also provide the opportunity to study ischemia-reperfusion (IR) damage of the intestine as described in **Chapter 6**. Although the rat PCIS remain viable until 5 hours incubation with respect to the maintenance of an intact epithelial lining, metabolism and transport capacity, their viability decreases after longer incubation and there are indications that PCIS suffer from IR induced damage during the preparation and culturing. Decreased ATP, induced inflammatory gene expression, and induced oxidative stress were studied in rat PCIS during incubation. Moreover, ischemic preconditioning and H2S preconditioning were used as an attempt to improve the reduced viability due to IR injury in PCIS.

Finally, the outcome of the work described in the chapter 3-6 is summarized and discussed in **Chapter 7**. In addition, suggestions based on our experiments and future perspectives for using PCIS as an *ex vivo* model to study intestinal toxicity are discussed.