Preface

Legislation in the area of safety testing of chemicals has drastically changed during the last decade. Thus, adopted in 2007, the European chemicals policy, commonly known as REACH (i.e., *r*egistration, *e*valuation, *a*uthorization and restriction of *ch*emicals), demands the safety assessment of thousands of chemical substances [1]. Clearly, the large-scale testing exercise imposed by the REACH regulation inherently requires high amounts of laboratory animals, not only raising serious ethical questions but also having a substantial economic impact. On the other hand, in compliance with the European Regulation 1223/2009, which became fully valid in 2013, animals can no longer be used for the toxicity testing of cosmetic products and their ingredients [2]. These clear-cut regulatory developments collectively illustrate that there is a ubiquitous need for alternative methods for the safety assessment of chemicals, such as in vitro testing platforms, which is in line with the 3R concept of Russell and Burch, calling for *r*efinement, *r*eduction, and *r*eplacement of animal experimentation [3]. In fact, the tendency for adopting nonanimal methods for toxicity testing is not only expected to affect other chemical areas in Europe in the near future, including the plant protection product and pharmaceutical fields, but also gains increasing importance in other parts of the world (e.g., the USA, Brazil, Canada, and Korea).

The liver fulfills a number of vital functions in the body, such as the control of lipid and carbohydrate homeostasis, the synthesis of various proteins, the storage of vitamins, the processing of bile, immunological defense, and the biotransformation of endogenous molecules and xenobiotics. Most of these functions are mediated by the hepatocytes, which are parenchymal cells that constitute as much as 80 % of the total liver mass [4]. Inherent to these critical tasks, in particular their biotransformation activity, hepatocytes form major targets for disease and systemic toxicity. For this reason, most attention has been traditionally paid, and is still being paid, to liver-based in vitro models in the area of 3R alternative methods for toxicity testing. A plethora of hepatic in vitro systems is currently available, ranging from hepatocyte subcellular fractions to whole isolated perfused livers [5, 6]. Among those, cultures of primary hepatocytes, especially of human origin, are generally considered as the gold standard in the field of liver-based in vitro modeling [6, 7]. Primary hepatocytes are typically isolated from freshly removed livers by means of a two-step collagenase perfusion technique [8]. As they are scarce, isolated human hepatocytes may be cryopreserved prior to cultivation and use [9]. Primary hepatocytes provide a good reflection of the hepatic in vivo situation for merely a couple days when properly cultured. Hence, their conventional cultures can only be used for short-term purposes. Indeed, long-term cultivation of primary hepatocytes is largely impeded by the progressive loss of the hepatocyte-specific phenotype both at the morphological and at the functional level. This so-called dedifferentiation process, which is initiated during the hepatocyte isolation procedure, can be counteracted, at least in part, by a number of classical cultivation strategies that try to restore the natural hepatocyte microenvironment in vitro as well as by a number of novel (epi)genetic approaches [5, 7, 10, 11]. Such optimized primary hepatocyte cultures are of great value, not only for long-term toxicity testing schemes but also for studying basic hepatocyte (patho)physiology. In this context, insight into the biological development of

hepatocytes has greatly increased in the last few years through in vitro differentiation of different types of stem cells into hepatocyte-like cells using a number of experimental approaches [12–15]. Along the same line, proliferative events can be mimicked in vitro either by cultivating primary hepatocytes in very specific conditions [16] or by using pathologically altered or artificially modified hepatocytes [17]. In addition, primary hepatocyte cultures have been proven ideal tools to study the biological counterpart of liver cell growth, namely hepatocyte cell death [18].

Functional hallmarks of cultured primary hepatocytes, such as metabolic competence, are frequently evaluated by monitoring the transcriptional and translational levels of liver-specific gene expression. Expression studies using transcriptomics and proteomics methodologies can indeed shed light onto the comprehensive suite of events at the most upstream regulatory level of liver-specific functionality. More recently, other disciplines have also entered the "-omics" arena, including metabonomics and epigenomics [6]. Such cutting-edge technologies can be equally applied for evaluating the response of cultured primary hepatocytes to toxic substances. Among those are several compounds which may trigger different types of drug-induced liver injury. Drug-induced liver injury is of high clinical concern, as it is the leading cause of acute liver failure. Furthermore, drug-induced liver injury is also of clear relevance to pharmaceutical industry, since it underlies the withdrawal of a considerable number of drugs during premarketing and postmarketing phases of drug development [19]. These various types of hepatotoxicity all have their own very specific morphological and functional characteristics that can be investigated in liver-based in vitro models, including primary hepatocyte cultures [6, 20].

The present book provides a state-of-the-art compilation of protocols and reviews to practically set up and apply primary hepatocyte cultures for research and screening purposes. In a first part, methods for isolating hepatocytes from liver tissue (*see* Chapter 1) and their cryopreservation (*see* Chapter 2) as well strategies for studying the different aspects of the hepatocyte life cycle in vitro are outlined. In particular, hepatocyte proliferation can be induced in vitro by using specific mitogens as cell culture medium additives for primary hepatocytes (*see* Chapter 3) or by using immortalized (*see* Chapter 4) or tumor-derived (*see* Chapter 5) hepatic cell lines. Following is a protocol on how to study and evaluate apoptotic cell death in cultured primary hepatocytes (*see* Chapter 6). Thereafter, the in vitro generation of hepatocyte-like cells from embryonic stem cells (*see* Chapter 7), adult stem cells (*see* Chapter 8), and induced pluripotent stem cells (*see* Chapter 9) is described. Considerable attention is subsequently paid to approaches to maintain the adult differentiated status in cultured hepatocytes, including the use of differentiation-promoting cell culture medium additives (*see* Chapter 10), the re-establishment of heterologous cell–cell contacts (*see* Chapter 11), the re-introduction of an extracellular matrix backbone (*see* Chapter 12), the setup of advanced liver bioreactors (*see* Chapter 13), chromatin remodeling (*see* Chapter 14), and the overexpression of genes that promote the liver-specific phenotype (*see* Chapter 15).

In the second part of the book, a number of routinely used and recently introduced in vitro techniques to monitor hepatocyte functionality and toxicity are presented. Global profiling at the transcriptomic (*see* Chapter 16), epigenomic (*see* Chapter 17), proteomic (*see* Chapter 18), and metabonomic (*see* Chapter 19) level can provide detailed mechanistic information on modifications in cellular pathways in primary hepatocyte cultures upon exposure to xenobiotics, whether or not of deleterious nature. The latter may affect critical liver-specific functions, which can be probed in vitro, namely biotransformation capacity (*see* Chapter 20), drug transporter activity (*see* Chapter 21), albumin secretion (*see* Chapter 22),

synthesis of blood coagulation factors (*see* Chapter 23), ammonia detoxification (*see* Chapter 24), and bile secretion (*see* Chapter 25). In the final section, protocols to evaluate toxic responses in liver-based in vitro models are presented, both general cytotoxicity (*see* Chapter 26) and cell death (*see* Chapter 27) as well as specific types of drug-induced liver injury, including cholestasis (*see* Chapter 28), steatosis (*see* Chapter 29), and hepatic fibrosis (*see* Chapter 30).

The current book is intended for basic and applied researchers, ranging from the undergraduate to the postdoctoral and professional level, in the area of pharmacology and toxicology, both in academic and industrial settings. It can be used by investigators familiar and unfamiliar with the field of liver-based in vitro modeling and testing.

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References

1. Regulation (EC) No. 1907/2006 of the European Parliament, of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation, Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC, repealing Council Regulation (EEC) No 793/93, Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC, Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC, 2000/21/EC. Off J Eur Union L396:1–849
2. Regulation (EC) No. 1223/2009 of the European Parliament, of the Council of 30 November 2009 on cosmetic products (recast). Off J Eur Union L342:59–209
3. Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, Co Ltd., UK
4. Arias IM, Alter HJ, Boyer JL, Cohen DE, Fausto N, Shafritz DA, Wolkoff AW (2009) The liver: biology, pathobiology. 5th edn. Wiley-Blackwell, Oxford
5. Vinken M, Vanhaecke T, Rogiers V (2012) Primary hepatocyte cultures as in vitro tools for toxicity testing: *quo vadis*? Toxicol In Vitro 26:541–544
6. Godoy P, Hewitt NJ, Albrecht U et al (2013) Recent advances in 2D, 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources, non-parenchymal liver cells, their use in investigating mechanisms of hepatotoxicity, cell signaling, ADME. Arch Toxicol 87:1315–1530
7. Fraczek J, Bolleyn J, Vanhaecke T et al (2013) Primary hepatocyte cultures for pharmacotoxicological studies: at the busy crossroad of various anti-dedifferentiation strategies. Arch Toxicol 87:577–610
8. Papeleu P, Vanhaecke T, Henkens T et al (2006) Isolation of rat hepatocytes. Methods Mol Biol 320:229–237
9. Hewitt NJ (2010) Optimisation of the cryopreservation of primary hepatocytes. Methods Mol Biol 640:83–105
10. Vinken M, Papeleu P, Snykers S et al (2006) Involvement of cell junctions in hepatocyte culture functionality. Crit Rev Toxicol 36:299–318
11. Elaut G, Henkens T, Papeleu P et al (2006) Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes, their cultures. Curr Drug Metab 7:629–660
12. Snykers S, De Kock J, Vanhaecke T et al (2011) Hepatic differentiation of mesenchymal stem cells: in vitro strategies. Methods Mol Biol 698:305–314
13. Snykers S, De Kock J, Rogiers V et al (2009) In vitro differentiation of embryonic, adult stem cells into hepatocytes: state of the art. Stem Cells 27:577–605
14. Al Battah F, De Kock J, Vanhaecke T et al (2011) Current status of human adipose-derived stem cells: differentiation into hepatocyte-like cells. Sci World J 11:1568–1581
15. Szkolnicka D, Zhou W, Lucendo-Villarin B et al (2013) Pluripotent stem cell-derived hepatocytes: potential, challenges in pharmacology. Annu Rev Pharmacol Toxicol 53:147–159

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