
Preface

In this book, we attempt to bring together the important issues and considerations we believe are needed in order to develop a workable, reliable, integrated testing strategy for the replacement of animals in toxicity testing regimes.

We begin the book with a review on “The past, present, and future of chemical risk assessment” by Alice Limonciel. She describes the history of the development of chemical testing and the evolution of chemical regulation. This process has had several key milestones. Among them were Elixir Sulfanilamide in 1937 and the thalidomide disaster in the late 1950s and early 1960s. The unfortunate use of the solvent diethylene glycol in Elixir Sulfanilamide caused the deaths of over a hundred people due to acute renal failure and introduced regulations where proof of safety of a compound was required to be shown before marketing. The thalidomide disaster introduced the necessity to test for reproductive toxicity. It is perhaps not surprising that these two examples were pharmaceuticals.

While testing is important for the chemical and cosmetic industry, the pharmaceutical industry is somewhat a special case as compounds are designed to be taken up, distributed, and have biological activity. Thus testing is a necessary and highly regulated part of drug development. However, just because we rigorously test compounds doesn’t mean we necessarily predict toxicities or a lack of them in humans. Individually, nonhuman mammals poorly predict human toxicity, and thus several species are used to cover predictive ground, unfortunately at the expense of specificity. Therefore, there is an inevitable loss of compounds which are toxic in animals but safe in humans. Thomas Hartung (contributor of Chap. 11) has pointed out that aspirin, one of the most widely used pharmaceuticals today, would have most likely not been brought to market if it had to pass through current preclinical testing regimes. Thus one of the main scientific rationales for developing in vitro alternatives is to improve on current animal-based testing regimes in the preclinical phase.

The ability to maintain cells outside the living body is documented as far back as 1885, when the zoologist Wilhelm Roux maintained embryonic chicken cells in a warm saline solution for several days. However, the true foundation of modern cell culture was arguably not until the mid 1950s when Eagle began to investigate the nutritional requirements of cells in culture [1, 2]. Already in 1959, Russell and Burch had realized the importance of cell culture as a real alternative to animal use, stating that “Mammalian tissue cultures have become one of the most important replacement techniques, and indeed one of the most important developments in biology” [3]. Since 1959, there has been a dramatic increase in the development and use of in vitro cell cultures which was mostly driven by technological advances in molecular biology such as polymerase chain reaction (PCR), transfection, and gene silencing. Primary cells and cell lines have now become extremely widely used tools. The improvements in cell immortalizations, such as telomerase overexpression [4], the development of high-content omic approaches, and the discovery of methods to make somatic cells pluripotent are continuing to push back the borders of in vitro research. These approaches are well suited to pharmacological and toxicological approaches and have great potential to increase our understanding of the molecular perturbations of chemicals and may eventually overtake animal studies as predictive tools.

The safety assessment of chemicals is a composite of hazard identification and risk of exposure. Thus for the pharmaceutical industry in particular, where the chemical is intended to enter the body and usually the circulation, all cells are potentially exposed making hazard identification of primary importance. If *in vitro* toxicity testing regimes are to replace whole animal tests for pharmaceutical development, they will have to represent all the major organs and tissues. While this might sound an impossible task, it could be more manageable by considering a tiered approach using the most commonly affected cells or tissues first. The first line are the liver and kidney, since, due to their respective roles in xenobiotic metabolism and excretion, they are exposed to and interact with a wide variety of chemical entities. So theoretically if a lead chemical demonstrated either hepatotoxicity or nephrotoxicity at concentrations close to their therapeutic range, they should be stopped at this stage. However, if this is not the case, other cell systems would then need to be tested. *In vitro* models for liver and kidney toxicity are discussed in Chaps. 2 and 4, respectively. The heart is obviously a vital organ and any compound that adversely affects its function could have very serious implications for health. Cardiotoxicity is the primary reason for postmarketing drug withdrawals and thus is of major interest for drug development [5]. The progress in the development of *in vitro* cardiac models is discussed in Chap. 3, and detailed protocols are provided. Neurotoxicity and injury to the blood brain barrier are also a major toxicological concern, particularly with the potential of chemical-induced injury to contribute to neurodegenerative diseases such as Alzheimer's and Parkinson's. The issues concerned and *in vitro* models available are detailed in Chaps. 6 and 7. The lung due to its involvement in blood oxygenation, metabolism, and the elimination of volatile substances is also an important toxicological target and is of special interest for drug delivery. The lung represents a selective barrier between the external and internal environments and is thus challenged on a permanent basis with air-borne pollutants including nanoparticles. *In vitro* models of the lung are reviewed in Chap. 5, while the special consideration of nanoparticles is addressed in Chap. 21. Xenobiotics have the potential to interfere with immune responses either by increasing or decreasing specific immune activity, and thus can lead to immunosuppression, sensitization, autoimmune disease, and may even promote cancer. The challenges associated with the development of immunotoxicity assays *in vitro* are discussed in Chap. 11.

The organs and tissues mentioned so far are important as their disturbance can lead to severe ill health and mortality. However, nonvital organs where quality of life can be severely impaired should also be considered for *in vitro* screening regimes. For example, many compounds, including aminoglycoside antibiotics, can cause permanent deafness, a situation which can have serious implications to life quality. The mechanism of ototoxicity and the *in vitro* models available are discussed in Chap. 9. Of special consideration for the cosmetic industry are the external physiological barriers and body surfaces, where cosmetics are often applied, for example the skin and eyes. Indeed the progress for the development of alternative nonanimal strategies has been most successful, so far, for dermal and ocular toxicity (Chaps. 8 and 10).

Many compounds either through direct action on DNA or indirect action, for example through chronic tissue injury, immunomodulation, and endocrine disruption, can cause cellular and tissue perturbations leading to the development of cancer. Thus, the carcinogenic potential of compounds is of critical importance for human safety. While several *in vitro* systems for testing genotoxicity are available, the identification of nongenotoxic carcinogens is more difficult. These issues are elaborated in more detail in Chap. 14.

In addition to effects of compounds on the intended individual, we also need to consider their impact on fetal development and reproductive potential. We now know that the

placenta is not an all exclusive barrier to the maternal environment. Certain chemicals, for example thalidomide, can cross this barrier, where they may cause serious adverse developmental effects. Chemicals, such as endocrine disrupters, may in addition reduce fertility which is a serious societal concern. Thus development and reproductive toxicity are important endpoints and are discussed in Chaps. 12 and 13.

As already mentioned, the fairly recent discovery of the possibility to induce pluripotency in somatic human cells (inducible Pluripotent Stem Cells, iPSC) [6], has the potential to revolutionize how we study human diseases and is likely to provide a plethora of new biological tools for pharmacological and toxicological investigations. Apart from providing a new source for primary cell culture, iPSC-derived target cells could form the first in vitro basis for studying population-based dynamics, genetic susceptibility, and idiosyncrasies. The development and use of iPSC for the major target organs is addressed in Chap. 15, while the use of iPSC and progenitor cells for neurodevelopmental toxicity is specifically reviewed in Chap. 16.

One of the major driving forces for the use of in vitro systems is their applicability to high-content analysis. Indeed the coupling of well-characterized relevant cell culture systems with powerful high-content, information-rich techniques such as transcriptomics, proteomics, metabolomics, and high-content imaging is pushing back the boundaries and allowing a true mechanistic understanding of molecular events (Chaps. 17 and 18) [7]. The use of these new technologies has provided us with a vast amount of mechanistic information on how cells function at a molecular level and how they deal with chemical and physiological stressors [8]. These types of experimental approaches are driving a new age in toxicological science where the focus is the discovery and elucidation of molecular mechanisms underlying chemical-induced cellular perturbations (Chap. 19). Indeed the OECD is promoting the development of the so-called “Adverse Outcome Pathways” (AOP) concept where a molecular initiating event, in which a chemical interacts with a biological target(s), is followed by a sequential series of events that ultimately result in an adverse outcome in an individual organisms or a population [9]. The elucidation of such molecular pathways relevant for adverse effects of compounds can lead us to the discovery of mechanistically anchored biomarkers. These biomarkers can be used to develop better predictive systems or may even be employed in clinical settings (Chap. 20).

A very important but often neglected aspect of in vitro toxicology is pharmacokinetics or toxicokinetics. Kinetics deals with how a test compound is altered by the system it is applied to. For an in vitro system, the available concentration of the compound can be decreased by binding to cell culture-ware such as a plastic cell culture dish, by binding to proteins in the cell culture medium, by evaporation and due to cellular uptake or cellular metabolism. The latter two points are critical in both in vitro and in vivo systems and are discussed in detail in Chap. 22. Knowing the actual concentration that cells can interact with, either by measurement as a free concentration in the cell medium or as a tissue concentration in the cell lysate, is crucial not only for experimental interpretation but also to extrapolate to the in vivo situation. Indeed, we must eventually extrapolate from in vitro to in vivo in order to establish safe exposure limits, which is after all the end goal of the exercise. These issues are dealt with in Chaps. 23 and 24.

In order to realize the vision of Russell and Burch and to go a step closer to animal free testing, we will require an integrated, systems biology approach, utilizing good cell culture practice [10], good laboratory practice, relevant and robust biological systems together with appropriate analytical tools and prediction models. Such an integrated strategy should be fit-for-purpose and need to be recognized and accepted by regulatory authorities. Thus

it is of utmost importance that scientists, industry, and regulators understand the needs of each other; only then can an integrated, tiered strategy based on *in vitro* techniques be put in place. In Chap. 25, the considerations in the development of *in vitro* toxicity testing methods intended for regulatory use are detailed.

In conclusion, the majority of the contributors of this book share our opinion that the use of animals for safety assessments is approaching its end of life and will eventually be phased out by more predictive human-derived *in vitro* systems and *in silico* approaches. What these *in vitro* systems will be like is uncertain, but we would be surprised if iPSCs were not an integral part of it, as this technology allows both human population-based screening and safety evaluation tailored to individuals. Finally, we were delighted to receive such a positive response from the experts we contacted and were very pleased that, without exception, each chapter was written with the high standards and expert insight that we hoped for. We are confident that this book has accomplished its goals and will be of benefit not only to students, scientists, and regulators working in the field of chemical safety assessment but also to the wider scientific audience.

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