Integrating toxicokinetics improves predictive value of primary rat hepatocytes

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The pharmacological as well as the potential toxicological effects are evoked by the effective dose of a drug at its site of action. Toxicokinetic (TK) properties are usually not characterised in in vitro toxicity models, which limits the use of these models. Thus, implementation of TK data into established in vitro systems holds great potential for improving the value of these systems. Here, TK data was integrated with multiple biological endpoints to characterise the long-term, repeat dose hepatocyte model for hepatotoxicity. For this purpose compounds with well described toxicities and kinetics in animals and humans were applied, namely Amiodarone, Chlorpromazine, Cyclosporine A and Ibuprofen. The long-term cell cultures were treated daily with two concentrations per compound for a period of 14 days. On day one, three and 14 sample collection was performed for global gene expression, metabonomic and proteomic analyses. Samples of supernatant, cells and plastic binding were collected at five different time points on the first and last day of treatment for kinetic analyses. Due to the metabolic competence of primary rat hepatocytes the amount of parent compound decreased continuously in the course of 24h after treatment start, notably to various extents on day 0 compared to day 13. Overall, valuable mechanistic information by comparing transcriptome, proteome and metabonome results was supported by TK knowledge. In conclusion, the primary rat hepatocyte model was improved involving TK data, hence the onset of pharmacological effects and the extrapolation of a NOEC (no observed effect concentration) for toxicity was facilitated.
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Risk assessment for dioxins in France based on a dynamic modeling of exposure

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Due to bioaccumulation, removal of dioxins from the human body is very slow, resulting in increasing levels of dioxins with exposure duration. Moreover, the environmental contamination by dioxins has varied greatly since their introduction in the 1930s. The objective of this work is thus to develop a method to assess the accumulated amount in the body (Body Burden) of the French population taking into account the change in exposure over time and the slow elimination from the human body. Dietary intakes of the French population are first calculated combining concentration data and consumption habit data. Then, current and future body burdens are estimated with a dynamic model which considers the accumulation due to successive dietary intakes and the elimination process between intakes. Moreover, in order to take into account the historic evolution of the exposure in this modeling, a Bayesian approach is used to fit a function which correct past exposure from biomonitoring data. Finally, present and future estimated body burdens are compared to different critical body burdens worked out from epidemiological human studies and from experimental studies using Bench Mark Dose modeling.

In 2009, the probability of exceedance of the various critical body burdens is significant and some individuals would remain highly exposed until 2030. However, a birth-cohort effect is demonstrated involving a reassuring downward timetrend for the risk related to dioxins. Simulations of body burdens of individuals born in 2010 suggest that dioxins levels in food would not be a future public health concern in France.
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Utilising chemiluminescent methods for animal-free toxicology tests

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The use of molecular endpoints to identify and classify potentially toxic compounds provides significant potential for high-throughput in vitro screening. We have developed a highly reproducible and robust method of direct quantification of gene expression based on chemiluminescence. Seven target genes were chosen for their ability to inform on toxicity and mechanism and assays for their expression were developed and optimised. To assess genotoxicity, p53, RAD51C and CSTA were selected after a study by Westerink et al (2010) showed that these targets provided good predictive indices of genotoxic potential in the group of compounds screened. Additionally, we have developed gene-based tests for a group of enzymes involved in steroidogenesis (CYP21, 19, HSD17B2). For the purpose of standardisation, an assay was also developed for beta-actin to be used in parallel with the other assays. Our assay method involves the direct quantitation of RNA transcripts by the use of chemiluminescent -labelled complementary probes and is capable of analytical sensitivity of 10-50 attomol of target with a routine coefficient of variance of less than 15%. In addition to their high sensitivity, these assays have the ability to distinguish between targets differing by a single base pair providing extraordinary specificity for the intended target. These tests are being applied in combination with cell culture technologies to evaluate potentially toxic compounds in a high throughput format. The exploitation of genetic endpoints will provide a basis for a rapid screening technology allowing accurate assessment of the mechanistic action of new drugs / chemicals.