

Lecture: strategies to reduce animal numbers for testing biologicals

Analysis of potential toxicity caused by genetically modified plants: a combination of *in vitro* methods as an alternative to feeding studies

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The approval of genetically modified plants (GMP) as food or animal feed is a major issue especially with regard to potential effects on human and animal health. While the admittance of drugs has to follow a strongly regulated process involving a variety of preclinical and clinical assays and tests, the rules for the approval of GMPs are in part still to be determined. In this study we have fed GMPs to rats according to OECD guideline 407. In addition, we have established a combination of *in vitro* assays using *ussing* chambers equipped with small intestine of pigs (slaughterhouse material) to simulate intestinal transport from mucosal to serosal side and hepatocyte cultures to assay for potential effects on hepatocellular metabolism and toxicity. We analyzed the synthesis of albumin as a marker of blood synthesis and anabolic function of the liver as well as the synthesis of urea as an example for a catabolic process in the liver. Several different GMPs were assayed *in vivo* and *in vitro*. For the *in vitro* assay, lyophilized potato tubers were dissolved in phosphate buffered saline and applied to the mucosal side of the transporting tissue. After 6 hours transport processes were stopped and probes from the serosal as well as the mucosal site were assayed in hepatocyte cultures derived from human, monkey, dog and rat. Dog and monkey hepatocytes were isolated from animals that either served as controls in animal experiments or were mercy killed due to terminal illness.

There was no indication, that the GMPs used in feeding studies influence the health or behaviour of the animals. Moreover, despite the fact that recombinant expressed protein was transported from the mucosal to the serosal side none of the GMPs did have any toxic effect on hepatocytes of the different species and, in addition, hepatocellular function as detected by albumin and urea release was not altered by GMPs in any of the species tested. The similarity of the *in-vivo* and the *in-vitro* results strengthens the assumption that, the combination of these two *in vitro* methods can be used as an alternative to animal feeding studies in order to determine potential toxicity of GMPs and their effect on liver metabolism. Of course further evaluation of the *in-vitro* systems by substances able to serve as positive controls has to be performed.

Keywords: hepatocyte cultures, ussing chamber, genetically modified plants, toxicity, metabolism, transport