Summary

Compounds of anthropogenic origin such as bisphenol A (BPA), but also naturally occurring phytochemicals such as the isoflavones daidzein and genistein are known to have estrogenic activity and can exert developmental and reproductive toxicity in animals at sufficiently high doses. There is concern that such chemicals, upon ingestion with food, may also lead to effective levels in humans and cause adverse effects by interfering with hormonally regulated processes (endocrine disruption). In the present work analytical methods for the determination of BPA, daidzein, equol and genistein in plasma, tissue and urine were developed, optimized and validated to further examine exposure, distribution and elimination of these endocrine active compounds and their metabolites.

BPA, a copolymer in polycarbonate plastics and food packing materials, is mainly ingested as dietary contaminant. Its distribution and transplacental transfer was investigated in a kinetic study in rodents: Upon i.v. administration of BPA to pregnant rats, the levels in plasma and in several maternal and fetal tissues were determined at various times after application. For the analysis of the samples, a liquid/liquid (l./l.) extraction method followed by GC-MS analysis was optimized and validated for BPA analysis in these biological matrices. The method was suitable for measuring reliably BPA concentrations in plasma and in tissues down to the limits of detection (LOD) of 14 ng/mL and 32 ng/mL (calculated according to DIN 32645). The recovery varied between 84% - 110 %, depending on tissue and concentration, and the inter-assay variations were calculated to 6 % and 10 % for plasma and liver.

The analysis of plasma and tissue samples from pregnant DA/Han rats, treated with 10 mg/kg bw BPA i.v. on day 18 of gestation, showed the following results: Soon (5 min) after dosing high plasma concentrations (3.8 µg/mL) were measured which declined within 2 h to the twentieth part of the initial value. Moreover, rapid metabolism of BPA by phase II enzymes to glucuronide conjugates was observed. Half-lifes of 0.34 h for the BPA-aglycon and 0.52 h for total BPA (conjugates) were calculated from plasma concentration time courses. In tissues, the highest concentrations occurred 20-30 min after injection, with values in falling order in: liver(dam) > kidney(dam) > uterus > placenta > liver(fetus) > fetus homogenate. In the course of the experiment peak values decreased quickly, and after 6 h only small parts of BPA were detected in the tissues. The rather similar concentration time courses in placenta and in fetal liver argue for an undisturbed transfer of BPA into the fetus. In summary, the results of this animal study point to a fast distribution of BPA in maternal and fetal tissues, yet indicate also an efficient conjugation and elimination [Moors *et al.*, 2006, Arch. Toxicol. 80: 647-655].

For studies on the uptake and bioavailability of the isoflavones daidzein and genistein which are mainly found in soy, we analyzed a) the isoflavone content of some pig feeds and b) isoflavone concentrations in pig blood (plasma samples) from groups of animals fed with the experimental diets. For the analysis of pig feed a method involving acid hydrolysation and ethanol extraction followed by micro HPLC-UV analysis with standard addition was used. The limits of detection were 10 µg/g for both daidzein and genistein (calculated via S/N = 3 procedure). For analysis of pig plasma, enzymatic hydrolysis and liquid/liquid extraction followed by GC-MS analysis was used. For this method limits of detection were 11 ng/mL and 9 ng/mL for daidzein and genistein (according to DIN 32645) and the recovery rates were between 72 – 121 %, depending on substance and concentration. The interassay variations were 5 - 15 %. Our analysis of pig plasma and pig feeds showed that, in the soy-free fed group almost no daidzein or genistein circulated in the plasma, whereas 10-20 fold higher plasma-concentrations were found in the standard-fed group, with an uptake of isoflavones of 2.1 mg/kg bw. Thus, the isoflavones were bioavailable. Moreover, the feeding study with gilts receiving about ~180 mg/day isoflavone showed no significant changes in a range of parameters when compared to soy-free fed animals [Kuhn *et al*., 2004, Arch Anim. Nutr. 58: 265-276].

To investigate human exposure against BPA, daidzein and genistein, a biomonitoring method based on solid phase extraction followed by GC-MS analysis was established which allows simultaneous determination of the analytes and their metabolites in small volumes of urine. For this method the application of a cooled injection system in solvent vent mode improved the limits of detection for BPA, equol, daidzein and genistein to 3, 4, 4 and 5 ng/mL; recovery rates varied depending on substance and concentration between 80 - 127 %. The interassay variations were 10 % for BPA, 9 % for daidzein, 15 % for equol and 18 % for genistein.

This method was then applied to an analysis of BPA, daidzein, equol and genistein levels in spot (7) and in 24 h urine samples (8) of volunteers on a typical German diet to estimate their exposure on the basis of excreted amounts. In the 24 h urines, mean values of 9, 98, 38 and 102 µg/day were calculated for BPA, daidzein, equol and genistein, respectively. To investigate the ranges of isoflavone intake and excretion, also urine samples collected from one volunteer on an isoflavone-free diet and after soy challenge were analyzed. No isoflavones were found in his urine after a period of 52 h on isoflavone-free diet, whilst the defined isoflavone intake of 12.9 mg daidzein and 25.2 mg genistein in soy protein (typical exposure with an Asian-type diet) resulted in high urinary concentrations of daidzein, equol and genistein, with

recoveries of 13 % of the dose for daidzein (or 37 % when equol is included) and 6 % for genistein in the 24 h urine [Moors *et al.,* submitted to Molec. Nutr. Food Res.].

Data obtained by applying the validated analytical methods to a kinetic study in rats and a small biomonitoring study provide new information useful in a comparative toxicological risk assessment for the environmental estrogens BPA, daidzein, and genistein: (a) The levels determined in urine samples of our German cohort indicate a low exposure to BPA and isoflavones with the typical Western-type diet. Exposure to BPA is clearly (at least 10-fold) lower than that to isoflavones. Taking into account that the part of an ingested dose which is excreted in urine is higher for BPA (>90%) than for the isoflavones (<40%), this difference becomes even more pronounced. (b) The animal study on the toxicokinetics of BPA analyzed by us as well as related studies by others with BPA and isoflavones indicate that due to rapid phase-II metabolism and excretion only a fraction of the estrogenic parent compound is bioavailable in the organism.In light of the known and low hormonal potency of the compounds and the low dietary exposure they are unlikely to present a practical risk to human health. Yet, infants whose phase II metabolism is not fully developed and who are exposed to high levels of isoflavones with a special soy-diet, are considered as risk group by some committees [CERHR 2006, COT 2003, SKLM of the DFG 1998].