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MNF Meetings Report

25 Years Food Chemistry and Toxicology at the University of Kaiserslautern

Michael Habermeyer, Sabine Guth and Gerhard Eisenbrand

Division of Food Chemistry and Toxicology, Department of Chemistry, University of Kaiserslautern, Germany

In September 2007 the Division of Food Chemistry and Toxicology in the Department of Chemistry, University of Kaiserslautern, celebrated its 25th anniversary. Its development from its foundation 25 years ago exemplifies the success of a pioneering concept bringing together the areas of Food Chemistry and Toxicology in research and teaching. It also became rapidly evident that the "Kaiserslautern school concept" met specific requirements of employers from food, chemical and pharmaceutical industries. Moreover, against the background of harmonisation and "REACH", there is an increasing European demand for well trained experts in regulatory toxicology and/or food safety assessment. It can be stated today that the concept of bringing together Food Chemistry/Technology and biomolecular/life science/toxicology research and teaching proficiency has spread, and there is an increasing number of chemical departments in Germany where similar structures have been or are being installed.

To celebrate the occasion, a scientific symposium entitled "Food Chemistry and Toxicology – Cornerstones of Life Sciences in Chemistry" was held in Kaiserslautern (Germany). Key aspects of the scientific programme comprised topics also representing major areas of interest of the division of Food Chemistry and Toxicology in the past quarter century. Internationally renowned experts followed the invitation to give presentations about their research.

Correspondence: Professor Gerhard Eisenbrand, Division of Food Chemistry and Toxicology, Department of Chemistry, Technical University of Kaiserslautern, Erwin-Schoedinger-Str. 52, 67663 Kaiserslautern, Germany
E-mail: eisenbra@rhrk.uni-kl.de
Fax: +49 631 205 3085

Abbreviations: **AA**, acrylamide; **Ah,** aryl hydrocarbon; **AhR,** aryl hydrocarbon receptor; **HBCDD,** hexabromocyclododecane; **NDELA,** *N*-nitrosodiethanolamine; **NHMOR,** *N*-nitroso-2-hydroxymorpholine; **OTA,** ochratoxin A; **PA,** pyrrolizidine alkaloids; **PBDE**, polybrominated diphenylethers; **PCB,** polychlorinated biphenyl; **TCCD**, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Richard N. Loeppky, Department of Chemistry, University of Missouri (USA), presented a lecture on "Mechanistic Studies on the Formation, Occurrence and Metabolism of Nitrosamines". *N*-Nitrosocompounds were identified to be potent carcinogens 50 years ago. Nitrosamines are environmental carcinogens occurring as contaminants in various consumer products, including food, cosmetic products, and rubber-based non-food products. They have also been found as pollutants at certain working places (*e.g.* in rubber, leather, chemical and metal cutting industry). Mechanistic studies on the formation, occurrence and metabolism of these compounds have been carried out worldwide. Moreover, sustained research activities directed towards prevention of human exposure have brought about a significant reduction.

From early times, basic research also markedly contributed to understanding the molecular mechanisms of the carcinogenic activity of *N*-nitroso compounds. *N*-Nitrosamines, such as *N*-nitrosodimethyl- or -diethylnitrosamine, undergo cytochrome P450 (CYP)-mediated hydroxylation at the α-C position. The resulting α-hydroxy compound dissociates rapidly into an aldehyde and the corresponding monoalkylnitrosamine, *i.e.* an alkyldiazonium intermediate. This strong electrophile reacts with cellular biopolymers, including DNA, causing formation of alkyl adducts at nucleophilic sites, such as O6 or N7 of guanine. These lesions may result in fixation of mutations. Investigations on the mechanism of metabolic activation of *N*-nitrosodiethanolamine (NDELA) and related β-hydroxynitrosamines have shown them to be bident carcinogens, because two fragments from the same molecule, glyoxal and a diazonium-derived alkylating agent, bind to DNA [1]. The metabolism of NDELA involves competitive oxidation of the 2-hydroxyethyl side chain in both the α- and the β-position. The latter process produces *N*-nitroso-2-hydroxymorpholine (NHMOR) through cyclization of the produced aldehyde. NHMOR is a direct acting mutagen which is further metabolized to glyoxal [2]. CYP 2E1-mediated oxi-

dation of NDELA occurs to yield NHMOR as well as 2-hydroxyethyl diazonium ion and glycolaldehyde. NDELA metabolism produces both glyoxal-guanine and O6-hydroxyethylguanine adducts in rat liver DNA after oral administration [3, 4]. Furthermore, reactions between α-amino acids, aldehydes and nitrite have been elucidated under simulated gastric conditions to yield *N*-nitroso-1,3-oxazolidine-5-ones (NOZ). These compounds, depending on their structure, exhibit activites similar to direct acting genotoxic agents in the comet assay and in mutagenicity tests.

Steven R. Tannenbaum, Massachusetts Institute of Technology (USA), gave a survey on the subject: "From Nitrite to Nitric Oxide: The Path to Discovery". Studies on endogenous formation of nitrosamines from dietary nitrite have led to a better understanding of this chemistry and how to prevent endogenous nitrosamine formation. In addition, they also have markedly driven the discovery of nitric oxide (NO) as an endogenous molecule. In the early seventies it was discovered that nitrite was found to be present in human saliva together with nitrate. Reduction of nitrate secreted into saliva by the oral microflora was an appropriate explanation, also triggering concern about the potential endogenous formation of *N*-nitroso compounds by interaction of salivary nitrite with dietary precursors in the acidic stomach [5]. More importantly, the discovery that the total amount of nitrate in body fluids (saliva, urine, sweat, feces) and excreta exceeded the amount of nitrate ingested, was unexpected and initiated fundamental research into the origin of endogenous nitrate. Until the seminal observation by the Tannenbaum group, it was believed that body nitrate just reflected the uptake *via* food and water. There was no scientific basis for the possibility that trivalent nitrogen could be oxidized in the organism to the level of nitrate by mammalian enzymes. The discovery that nitrate excretion by a human volunteer increased during an episode of intestinal infection presented an unexpected clue [6]. The finding was confirmed by an animal experiment in rats treated with lipopolysaccharides to induce an inflammatory response. Subsequently, Marletta and co workers discovered an inducible enzyme that generated NO from arginine, NADPH and O_2 which was baptized nitric oxide synthase (NOS) [7, 8]. Other NOS isoforms, including endothelial and neuronal NOS, were discovered by other groups. Today, NO is recognized as a second messenger molecule, important in signalling pathways operative in various biological functions, including relaxation of smooth muscle cells, neurotransmitter activity in the central/peripheral nervous system as well as macrophage mediated defense activity. Endogenously formed NO can also act as a precursor for further reactive nitrogen species. Reaction with the superoxide anion radical results in the formation of peroxynitrite that dissociates in $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals, the latter being converted to nitrate.

Lorenz Poellinger from the Karolinska Institute Stockholm (Sweden) presented "Mechanism of signal transduction and gene regulation by the dioxin receptor". The aryl hydrocarbon receptor (AhR) functions as a ligand-activated transcription factor, regulating transcription of a battery of genes encoding xenobiotic metabolizing enzymes. It is activated by a series of environmental pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated dioxins and biphenyls, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCCD). It belongs to the basic helix-loop-helix (bHLH)/Per-ARNT-Sim domain (PAS) family of transcription factors that also contains further gene regulatory proteins responsive to environmental factors. In contrast to these well characterized exogenous ligands of the AhR, there is as yet no conclusive information about respective endogenous ligands. Binding of a ligand induces translocation of the ligand-receptor complex into the nucleus by interaction with the Ah receptor nuclear translocator (ARNT). The active transcription factor dimer binds to xenobiotic responsive elements (XRE) in cognate DNA promoter elements and induces the expression of several gene families, including metabolizing enzymes of phase I (cytochrome P450 1A1, 1A2, 1B1) and phase II (glutathion-S-transferase, UDP-glucuronosyltransferase). Information on target genes that are not drug metabolizing enzymes is largely absent. In order to study the mechanism behind the toxicity of ligands of the AhR a transgenic mouse model expressing a constitutively active Ah receptor (CA-AhR) has been created [9]. Further mechanistic studies appear mandatory to more precisely understand the regulation of the AhR in the absence or presence of ligands.

Martin van den Berg, Utrecht University (The Netherlands), informed on "Recent developments in the toxicology of brominated flame retardants with special emphasis on polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCDD)". Brominated flame retardants, especially polybrominated diphenylethers have gained increasing scientific and public interest due to their occurrence in the environment, in humans and wildlife [10]. PBDEs have a non planar configuration and therefore might show some similarity to non dioxin, ortho substituted polychlorinated biphenyls (PCBs) in terms of mechanism of action. Moreover, a number of non Ah receptor-mediated endpoints, including endocrine, neurotoxic and neurobehavioral effect have come into focus. Although distinct structure activity relationships are not obvious, similarities between effects of non dioxin ortho subtituted PCBs and PBDEs appear evident from a number of recent studies, suggesting the use of a combined risk assessment for both groups.

In addition, endocrine related effects have been reported for PBDE metabolites, especially hydroxylated metabolites (OH-PBDEs). This applies to interactions with the estrogen receptor (ER), the thyroid hormone axis (transthyretin, TTR) and the steroidogenic enzymes CYP17 and 19 (aromatase). Thus, *in-vivo* studies that include sensitive life stages appear necessary and should be supported by appro-

priate mechanistic studies in human cells. Relating dose-response relationship to actual human blood/tissue concentrations might provide information relevant to adequate risk assessment. Some current data are available in foods [11-14] and humans [15-20].

Wolfgang Dekant, University of Wuerzburg (Germany), presented "Molecular Mechanism of Ochratoxin A Toxicity and Carcinogenicity". The mycotoxin Ochratoxin A (OTA) is a long known and widespread contaminant produced by several *Aspergillus* and *Penicillum* species. OTA is a potent nephrotoxic carcinogen that has been discussed as a potential risk factor related to Balkan endemic nephropathy. In rodents, OTA is nephrotoxic and induces renal tumours, but there is a significant gender and species difference and the mechanism of carcinogenicity by OTA is still not fully understood [21]. Some data in the literature are supportive for a genotoxic mechanism and for formation of DNA adducts by OTA. Other publications have provided evidence arguing against a direct genotoxic mechanism [22]. OTA is not mutagenic in bacterial mutagenicity tests but genotoxic effects have been observed in some mammalian cell systems. Approaches using radiolabeled OTA and liquid scintillation counting failed to show OTA-DNA adducts and these negative findings were confirmed by accelerator mass spectrometry, an ultrasensitve analytical method of ^{14}C carbon detection.

OTA has been shown to potently induce oxidative DNA damage in kidney cells *in-vitro* and *in-vivo* and this might well be causally related to malignant transformation. Moreover, recent evidence suggests that OTA might also interfere with microtubuli dynamics and mitotic spindle function and might cause aberrant mitosis, thus giving rise to cytogenetic abnomalities.

Doris Marko, University of Karlsruhe (Germany), portrayed "New research avenues in food chemistry". Food chemistry has witnessed a significant change in recent years, from an area of research focussing on analytical chemistry into a life science-driven science. Although still chemistry-driven with respect to elucidation of chemical processes during all stages of food production and processing, studies on physiological and health significance of such chemical processes have come much more into focus, bringing about an interdisciplinary approach supported by chemistry, molecular nutrition and toxicology. The increasing interest in health and well-being and a tremendous demand for products with bioactive properties resulted in an expanding market for functional food and food supplements [23].

As one example anthocyanins have been associated with a broad spectrum of beneficial health effects. However, the underlying mechanisms of action are still largely unknown. Biological activites have been reported mainly for the respective aglycons such as delphinidin, cyanidin or malvidin. More recently, complex berry extracts were also found to inhibit the activity of a broad spectrum of cellular receptor tyrosine kinases. Moreover, such berry extracts showed substantial inhibitory properties towards human topoisomerases I and II, similar to the activity of certain anthocyanins [24]. The interference with topoisomerases was shown to be limited to the suppression of the binding of topoisomerases to DNA. Thus, such compounds act as pure catalytic inibitors. In combination with therapeutically used topoisomerase poisons, berry extracts were found to modulate the DNA-damaging properties of such anticancer drugs [24, 25]. Further studies are required to elucidate whether enhanced intake of respective food supplements might pose the risk to compromise the therapeutic outcome of toposiomerase inhibitors.

The oral presentations were complemented by poster contributions, dealing to a major extent with potential beneficial health effects of diets containing fruits, vegetables or beverages, *e.g*. fruit juices or coffee.

Enhanced intake of secondary plant metabolites such as polyphenols/flavonoids appears to be associated with health benefits, for example prevention of cancer or cardiovascular disease [26]. One study reported a beneficial influence of apple pulp and cloudy apple juice on chemically induced inflammation in the colon of rats. Another study investigated the reduction of poly(ADP-ribosyl)transferase 1 (PARP1) transcription by phenolic apple juice extracts in human colon cancer cell lines, an effect suggested to improve the recovery of cells from oxidative cell damage, since extensive PARP activation might cause depletion of cellular ATP.

In an intervention study with patients on hemodialysis, marked reduction of oxidative cell damage and enhancement of oxidative defense in blood and lymphocytes after intake of a anthocyanin/polyphenolic rich fruit juice was observed [27].

In addition, studies have been presented confirming the contribution of the intestinal microflora to metabolism. Hydrolysis of polyphenol glucosides depended on the microflora and was abrogated by application of antibiotics [28]. An *ex vivo* ileo-/colostoma model was introduced as a promising tool to further investigate the intestinal transit and metabolism of various physiologically active compounds [29]. Another contribution presented evidence that a diet rich in products of the Maillard reaction was protective against Low Density Lipoprotein oxidation [30]. Furthermore, the polyphenol-mediated generation of peroxides in human colon carcinoma HT-29 cells and the influence on cellular redox markers has been investigated. Some presentations showed the analysis and characterization of compounds from plant material, such as red clover based supplements [31] and extracts from *Iris germanica*. Another group of poster contributions was related to contaminants in the food chain. Some presentations were devoted to the mechanism of TCDD-mediated liver tumour formation. No support was found for the hypothesis that estradiol-dependent redox cycling is relevant for carcino-

genicity. Rather, AhR-mediated induction of CYPs appeared as potential source for the formation of reactive oxygen species [32]. Furthermore, a presentation referred to the influence of TCDD on the induction of apoptosis. In contrast to cDNA-microarray experiments in mouse liver, in primary cultures of rat hepatocytes TCDD seemed not to induce the anti-apoptotic gene bcl-xL [33].

Another study investigated the cytotoxic and apoptotic effects of fumonisin B1 in primary rat hepatocytes. Only slight cytotoxicity was observed in concentrations up to 100 μM whereas the activity of the apoptotic key enzyme caspase-3 was increased dose-dependently [34].

Extending previous studies on the cytotoxicity, metabolism and cellular uptake of deoxynivalenol [35], a distinct cytotoxic effect was demonstrated for this mycotoxin in human primary hepatocytes and the activity of caspase-3 was found enhanced.

Lipophilic and persistent contaminants such as certain brominated flame retardents, including HBCDD or a technical mixture of polybrominated diphenylethers have also been investigated. It was shown that these compounds induce CYP3A4 *via* activation of pregnan X receptor in primary rat hepatocytes and in rat or human hepatoma cell lines [36].

A new reliable analytical method to determine pyrrolizidine alkaloids (PA) was presented, based on solid-phase extraction with subsequent silylation and capillary gas chromatography-MS. About 10% of commercially available honey samples (n = 216) contained PA at an average level of 0.1 μg/g [37].

Some poster presentations dealt with potentially toxic compounds formed during heating of food such as acrylamide (AA) or furan. One study investigated the influence of the food matrix on the bioavailability of AA administered in french fries, gingerbread and drinking water, and its biological effects in rats. It appeared that the food matrix did not affect the bioavailability to a major extent, as compared to intake through drinking water. Another study investigated the disappearance of AA during coffee storage and showed that AA irreversibly binds to coffee matrix constituents. First approaches towards cell culture experiments to evaluate cytotoxicity of furan reported on difficulties due to its rapid volatilization.

One study addressed the influence of technological processes on food constituents, especially the appearance of oxidative damage of milk proteins in heated raw milk or industrially produced infant formulae.

Finally, some novel mechanistic approaches towards targeted cancer therapy were presented. Structure -activity relationships of novel therapeutic compounds related to indirubin [38, 39] were discussed.

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[1] Loeppky, R. N., Goelzer, P., Microsome-mediated oxidation of *N*-nitrosodiethanolamine (NDELA), a bident carcinogen. *Chem. Res. Toxicol.* 2002, *15,* 457 – 469.

[2] Loeppky, R. N., Sukhtankar, S., Gu, F., Park, M., The carcinogenic significance of reactive intermediates derived from 3-acetoxy- and 5-acetoxy-2-hydroxy-*N*-nitrosomorpholine. *Chem. Res. Toxicol*. 2005, *18*, 1955 – 1966.

[3] Loeppky, R. N., Ye, Q., Goelzer, P., Chen, Y., DNA adducts from *N*-nitrosodiethanolamine and related beta-oxidized nitrosamines *in vivo*, (32)P-postlabeling methods for glyoxal- and O(6)-hydroxyethyldeoxyguanosine adducts. *Chem. Res. Toxicol*. 2002, *15,* 470 – 482.

[4] Dennehy, M. K., Loeppky, R. N., Mass spectrometric methodology for the determination of glyoxaldeoxyguanosine and O6-hydroxyethyldeoxyguanosine DNA adducts produced by nitrosamine bident carcinogens. *Chem. Res. Toxicol*. 2005, *18,* 556 – 565.

[5] Tannenbaum, S. R., Sinskey, A. J., Weisman, M., Bishop, W., Nitrite in human saliva. Its possible relationship to nitrosamine formation. *J. Natl. Cancer. Inst.* 1974, *53,* 79 – 84.

[6] Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D., Bruce, W. R., Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science*, 1978, *200,* 1487 – 1489.

[7] Marletta, M. A., Yoon, P. S., Iyengar, R., Leaf, C. D., Wishnok, J. S., Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochemistry.* 1988, *27,* 8706 – 8711.

[8] Hevel, J. M., White, K. A., Marletta, M. A., Purification of the inducible murine macrophage nitric oxide synthase. Identification as a flavoprotein. *J. Biol. Chem.* 1991, *266,* 22789 – 22791.

[9] Brunnberg, S., Andersson, P., Lindstam, M., Paulson, I., *et al*., The constitutively active Ah receptor (CA-AhR) mouse as a potential model for dioxin exposure – Effects on vital organs. *Toxicology* 2006, *224*, 191 – 201.

[10] Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M. *et al*., The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences* 2006, *93*, 223 – 241.

[11] Van Leeuwen, S. P. J., de Boer, J., Brominated flame retardants in fish and shellfish – levels and contribution to dietary exposure of Dutch citizens to HBCD. *Mol. Nutr. Food Res*. 2008, *52*, 194 – 203.

[12] Fernandes, A., Dicks, P., Mortimer, D., Gem, M., *et al*., Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: Methodology, occurrence and human dietary exposure, *Mol. Nutr. Food Res*. 2008, *52*, 238 – 249.

[13] Schecter, A., Harris, R., Shah, N., Musumba, A., Päpke, O., Brominated flame retardants in US food. *Mol. Nutr. Food Res*. 2008, *52*, 266 – 272.

[14] Ashizuka, Y., Nakagawa, R., Hori, T., Yasutake, D., *et al.,* Determination of flame retardants and brominated dioxins in fish collected from three regions of Japan. *Mol. Nutr. Food Res*. 2008, *52*, 273 – 283.

[15] Fängström, B., Athanassiadis, I., Odsjö, T., Noren, K., Bergman, A., Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers 1980 – 2004. *Mol. Nutr. Food Res*. 2008, *52*, 187 – 193.

[16] Thomsen, C., Knutsen, H. K., Liane, V. H., Froshaug, M., *et al*., Consumption of fish from a contaminated lake strongly affects the concentrations of polybrominated diphenyl ethers and hexabromocyclododecane in serum. *Mol. Nutr. Food Res.* 2008, *52*, 228 – 237.

[17] Bakker, M. I., de Winter-Sorkina, R., de Mul, A., Boon, P. E., *et al*., Dietary intake and risk evaluation of polybrominated diphenyl ethers in the Netherlands. *Mol. Nutr. Food Res*. 2008, *52*, 204 – 216.

[18] Knutsen, H. K., Kvalem, H. E., Thomsen, C., Froshaug, M., *et al*., Dietary exposure to brominated flame retardants correlates with male blood levels in a selected group of Norwegians with a wide range of seafood consumption. *Mol. Nutr. Food Res*. 2008, *52*, 217 – 227.

[19] Sioen, I., Bilau, M., Verdonck, F., Verbeke, W., *et al*., Probabilistic intake assessment of polybrominated diphenyl ethers and omega-3 fatty acids through fish consumption. *Mol. Nutr. Food Res*. 2008, *52*, 250 – 257.

[20] Antignac, J. P., Cariou, R., Maume, D., Marchand, P., *et al*., Exposure assessment of fetus and newborn to brominated flame retardants in France: preliminary data. *Mol. Nutr. Food Res*. 2008, *52,* 258 – 265.

[21] Pfohl-Leszkowitz, A., Manderville, R. A., Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Mol. Nutr. Food Res*. 2007, *51*, 61 – 99.

[22] Turesky, R. J., Perspective: ochratoxin A is not a genotoxic carcinogen. *Chem. Res. Toxicol.* 2005, *18*, 1082 – 1090.

[23] Eisenbrand, G., Evaluation of food supplements containing other ingredients than vitamins and minerals – Opinion of the senate commission on food safety (SKLM) of the German research foundation (DFG)-(shortened version). *Mol. Nutr. Food Res*. 2007, *51*, 1300 – 1304.

[24] Habermeyer, M., Fritz, J., Barthehnes, H. U., Christensen, M. O. *et al*., Anthocyanidins modulate the activity of human DANN topoisomerases I and II and affect cellular DNA integrity. *Chem. Res. Toxicol*. 2005, *18*, 1395 – 1404.

[25] Kern, M., Fritz, J., Marko, D., Delphinidin interferes with the DNA-damaging properties of the topoisomerase II poisons doxorubicin and etoposide in human colon carcinoma cells. *Toxicology Letters* 2007, *172,* S196 – S196.

[26] Schäfer, S., Baum, M., Eisenbrand, G., Dietrich, H., *et al*., Polyphenolic apple juice extracts and their major constituents reduce oxidative damage in human colon cell lines. *Mol. Nutr. Food Res*. 2006, *50*, 24 – 33.

[27] Albert, T., Rath, F. W., Dietrich, T., Will, H., *et al*., Anthocyanin/polyphenolic rich fruit juice reduces oxidative cell damage in an intervention study with patients on hemodialysis. *Naunyn-Schmiedebergers Arch. Pharmacol*. 2007, *375*, 95 – 95.

[28] Knaup, B., Kahle, K., Erk, T., Valotis, A., *et al*., Human intestinal hydrolysis of phenol glycosides – a study with quercetin and p-nitrophenol glycosides using ileostomy fluid. *Mol. Nutr. Food Res*., 2007, *51*, 1423 – 1429.

[29] Knaup, B., Kempf, M., Fuchs, J., Valotis, A., *et al*., Model experiments mimicking the human intestinal transit and metabolism of D-galacturonic acid and amidated pectin. *Mol. Nutr. Food Res*. 2008, *52*, DOI: 10.1002/mnfr.200700510, *this issue*.

[30] Dittrich, R., El-Massry, F., Kunz, K., Rinaldi, F., *et al.*, Maillard reaction products inhibit oxidation of human low-density lipoproteins in vitro, *J. Agric. Food Chem.* 2003, *51*, 3900 – 3904.

[31] Surtz, M., Lander, V., Schmid, W., Winterhalter, P., Preparative isolation of isoflavones from soy and red clover. *Mol. Nutr. Food Res*. 2006, *50*, 356 – 361.

[32] Göttel, M., Severin, I., Chagnon, M. C., Schrenk, D., Effects of estradiol on TCDD-induced oxidative stress in hepatoma cells. *Toxicology Letters* 2007, *172*, S229 – S230.

[33] Knerr, S., Schrenk, D., Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in experimental models. *Mol. Nutr. Food Res*. 2006, *50*, 897 – 907.

[34] Hecker, D. C., Schrenk, D., Cytotoxic and apoptotic effects of fumonisin B1 in cultured rat hepatocytes. *Toxicology Letters* 2007, *172*, S45 – S46.

[35] Königs, M., Lenczyk, M., Schwerdt, G., Holzinger, H., *et al*., Cytotoxicity, metabolism and cellular uptake of the mycotoxin deoxynivalenol in human proximal tubule cells and lung fibroblasts in primary culture. *Toxicology* 2007, *240*, 48 – 59.

[36] Germer, S., Van der Ven, L., Piersma, A. H., Schrenk, D., Effect of hexabromocyclododecane (HBCDD), a flame retardant, on expression of cytochrome P450 enzymes in rat liver. *Naunyn-Schmiedebergers Arch. Pharmacol*., 2005, *371*, R109 – R109.

[37] Kempf, M., Beuerle, T., Bühringer, M., Denner, M., *et al*., Pyrrolizidine alkaloids in honey: Risk analysis by gas chromatography-mass spectrometry. *Mol. Nutr. Food Res*. 2008, *52*, DOI: 10.1002/mnfr.200800051.

[38] Jautelat, R., Brumby, T., Schafer, M., Eisenbrand, G., *et al*., From the insoluble dye indirubin towards highly active, soluble CDK2-inhibitors. *Chembiochem* 2005, *6*, 531 – 540.

[39] Jakobs, S., Merz, K. H., Vatter, S., Eisenbrand, G., Molecular targets of indirubins. *Int. J. Clin. Pharmacol. Ther.* 2005, *43*, 592 – 594.

