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Publishable Final Activity Report

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Publishable Final Activity Report

In a recent investigation, the U.S. Food and Drug Administration (FDA) identified the chemical furan in a variety of food items that undergo heat treatment. Furan is a potent hepatotoxicant and liver carcinogen in rodents.

Although data on human intake of furan is limited, it appears that there is a relatively narrow margin between human exposure and doses which cause liver tumors in rodents, suggesting that the presence of furan in food may present a potential risk to human health. However, the presently available data on furan toxicity is insufficient to perform a risk assessment and more research regarding the mechanism of furan carcinogenicity is needed.

The project addressed modes of action for tumor induction in liver by the food contaminant furan, which is formed by processing of food resulting in widespread human exposure. There is uncertainty regarding the relevance of tumors induced in rodents to human risk assessment because the mechanisms are unclear. The research is addressing the role of DNA and protein binding of furan, oxidative DNA damage, non-genotoxic alteration of proliferation and apoptosis, cytogenetics and cytotoxicity in furan-mediated liver toxicity and carcinogenicity. A combination of *in vivo* and *in vitro* systems, analytical chemistry, cell biology and “omics” technologies was applied. In rodents *in vivo*, extent and dose-dependence of covalent binding to DNA, cytogenetic changes and geno- and cytotoxicity in target cells in the liver were addressed after oral administration of furan. In addition, the induction of oxidative DNA-modifications and mechanisms of mutations have been investigated in genetically modified rodent models. These *in vivo* studies characterize the mode of action of furan and also address irreversible metaplasia, changes in cell signaling and inflammation. The interaction of these effects with possible genetic changes in liver cells including aspects of forced cell proliferation have been included. The *in vivo* work has been complemented by studying mode of mutation of furan and its metabolite cis-2-butene-1,4-dial in cell culture models resembling the target cells. The content of furan in food was determined and human exposures was assessed using probabilistic modeling. Mechanisms of formation of furan in food may open ways to reduce exposures. The results provide data on the mode-of-action of furan induced liver carcinogenesis as a basis for a conclusive assessment of health risks in humans due to dietary exposure. The combination of these findings provide a risk/benefit analysis and a scientific basis to justify limits for human furan exposures.

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Objectives

Since the mechanisms of carcinogenic activity of furan in rodents are not well understood, the objectives of this project were to generate relevant mechanistic information as a support for the ongoing risk assessment of human furan exposures with food. The importance of mode-of-action research on furan is underscored by the comparatively small difference in the estimated human exposures and the doses of furan, which cause carcinoma in the liver of experimental animals.

A detailed elucidation of genotoxic and non-genotoxic mechanisms and their possible dose-response relationships and interconnectivity were of fundamental importance for a reliable risk assessment.

The final outcome of the project was a careful assessment of the genotoxic potential of furan in the target cells *in vivo* for carcinogenicity, generation of information on the potential relevance of DNA lesions and the tissue environment changes that may exacerbate mutations. The methods applied included state-of-the-art analytical procedures to quantify furan induced DNA-adducts in very low concentrations and other DNA-damage or cytogenetic changes, biomarkers, gene-arrays and modern cell biology to characterize furan induced changes in cellular function in the target cells of carcinogenesis in relevant species.

Following major objectives were addressed in this project:

1. Characterization and quantitation of DNA- and protein binding of furan in liver of rats and mice over a wide dose range. Both responses after single and after repeated exposures were assessed. (WPs 1.1 and 1.2)
2. Analysis of biomarkers of toxicity, genotoxicity and epigenetic changes affecting gene expression, tissue structure changes and cell proliferation in target cell populations of furan in order to elucidate cell-specific mechanisms. Again, special attention were given to determine the dose dependence of the effects. (WPs 2.1. and 2.2)
3. Assessment of the genotoxic and clastogenic potential of furan in rodent liver by comet assay, cytogenetics and biomarkers of genetic damage. (WPs 3.1 and 3.2)
4. Assessment of the role of the accumulation of oxidative DNA damage in the mechanism of neoplastic transformation by means of a genetically modified mouse models. (WP 4)
5. Detailed analysis of gene expression changes. (WP5)
6. Characterization and quantitative assessment of genetic changes induced by furan and cis-2-butene-1,4-dial in mammalian cells with special consideration on mechanisms for induction of gene mutations and cytogenetic changes. (WPs 6.1, 6.2 and 6.3)
7. Characterization of furan and cis-2-butene-1,4-dial induced effects on toxicity parameters, and DNA-damage in cell culture models for the specific target cells of furan in order to elucidate cell-specific mechanisms. (WP 7)
8. Analysis of furan in food as a better basis for exposure assessment from food and mechanisms of formation during food processing. (WPs 8.1 and 8.2)
9. Provision of a risk assessment of furan in food. (WP 9)

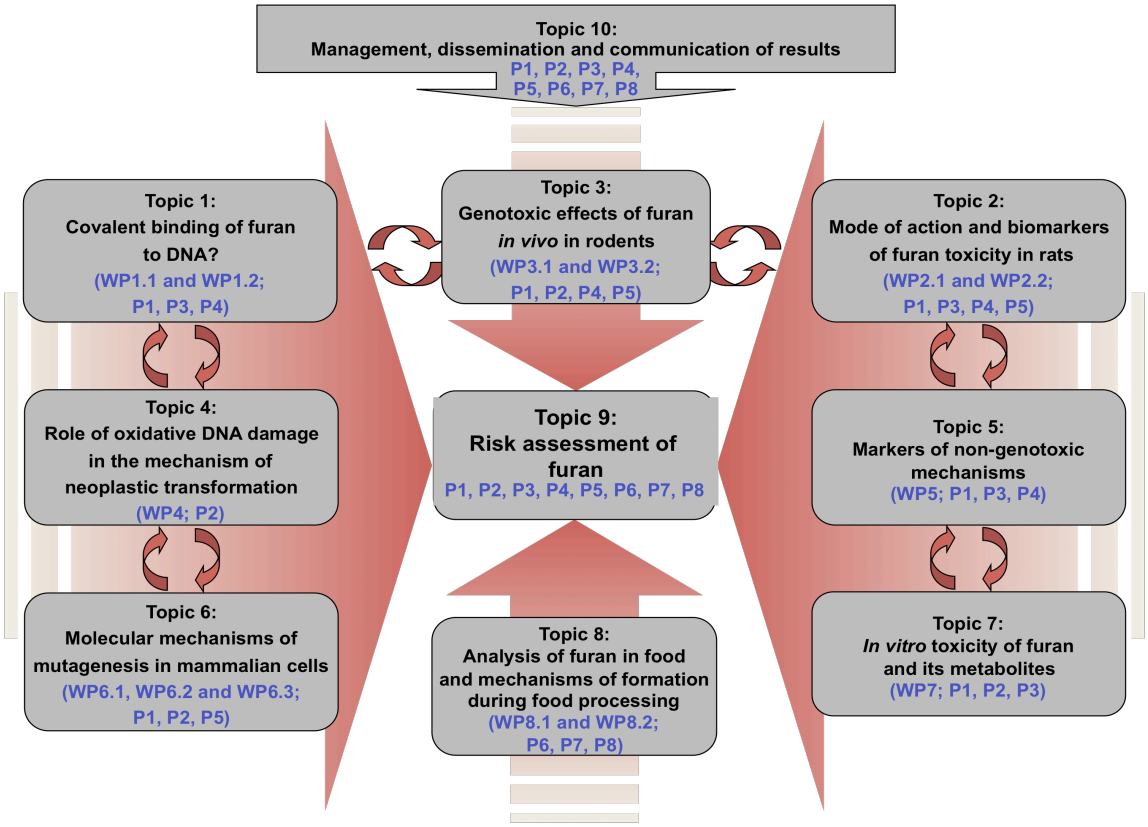

Fig. 1: Graphical presentation of the components showing their interdependencies

Work performed

In the first year, research activities were devoted to performing a 28-day repeated dose study with oral dosing of furan as a central repository for tissues to be analysed. In addition, study conditions regarding both in vivo and in vitro genotoxicity studies were optimized and preliminary results were generated. A database on levels of furan reported in food was assembled and mechanistic studies to investigate mechanisms of formation of furan in food were performed using stable isotope labeled precursors.

A Web-site (http://www.furan-ra.toxi.uni-wuerzburg.de/), which is regularly updated with results (deliverables and presentations presented by partners at the meetings) has been created. The web-site contains a public area and a password protected area for project participants, EC-representatives and external advisors.

In the second reporting period following work was performed:

To assess DNA-binding of furan, an in vivo study was conducted in which male Fischer rats were treated with ^{14}C-furan at a known carcinogenic dose and at a dose close to human exposure. DNA was extracted from livers of furan treated animals and analysed by accelerator mass spectrometry.

Subacute liver toxicity of furan in rats was characterized regarding changes in histopathology, clinical chemistry, metabonomics, genotoxicity, as well as non-genotoxic alterations in DNA-methylation, gene expression and cell proliferation.

The genotoxic activity of furan in B56C3F1 mice was investigated. Toxicity and genotoxicity end-points were evaluated in spleen and liver of animals receiving furan by the oral route along a four weeks period. Genotoxicity end-points were also assessed in animals receiving furan as an acute high dose.

In addition parallel measurements of DNA oxoG were performed in several organs of these Furan-treated mice. To investigate whether furan exposure increased oxidative DNA damage, C57BL/6 mice defective in the base excision repair *Ogg1* gene were also used. Experiments were performed in *Ogg1*$^{-/-}$ and wild-type mice using the same protocol of furan exposure. DNA 8-oxoG levels were measured in several organs and possible correlations with Furan-induced liver toxicity and genotoxicity were analysed.

Mutagenesis induced by furan and its metabolite cis-2-butene-1,4-dial were investigated in mammalian cells in culture at two different gene loci (i.e. hprt and tk). In parallel, DNA single strand breaks (ssb) and 8-oxoG levels were measured. The expression of a set of DNA repair genes was also monitored.

Two immediate precursors in the formation of furan during heat treatment of food were synthesized and their conversion into furan was investigated Strategies such as use of antioxidants or inert atmosphere were investigated to suppress lipid oxidation and hence reduce furan formation in food.

β-carotene and retinol were investigated as new highly potential precursors of furan. Enhancement of furan formation from unsaturated lipids in the presence of several prooxidants such as ascorbic acid, b-carotene, and transition metals was evaluated. Kinetics of furan formation was studied in food systems (pumpkin puree, carrot and orange juice) Activation energies were determined from obtained kinetic data.

Over one hundred food samples from the categories carrot and prune juices, nutrition drinks and bakery products were collected and analysed on furan levels. Attempts have been made to correlate the furan levels found with the ingredients and the production processes.

In the third reporting period following work was performed:

P1: Work performed in the third reporting period involved characterization of radioactive compounds to distinguish between metabolic incorporation and covalent binding and the design of a scheme of metabolic pathways leading to biliary metabolites of furan.

P2: The immunofluorescent detection of foci of phosphorylated histone H2AX (γ-H2AX), together with a radiation-modified comet protocol, were applied to investigate the formation of DNA-DNA crosslinks in mouse liver and spleen cells following acute and subacute furan administration.

A transcriptomic analysis on selected toxicity and DNA damage response genes in mouse liver was performed after 4 weeks exposure to a carcinogenic furan dose.

The organ specificity of oxidative DNA damage associated with Furan exposure was investigated in a set of experiments performed in C57BL/6 mice. In addition the possible relationship between 8-oxoG accumulation in the liver and the inflammatory response in this organ was explored by immunohistochemistry. Finally Furan-induced chromosomal damage in splenocytes was demonstrated to be independent from induction of oxidative DNA damage to the spleen.

Final data were obtained on the mutagenic potential of furan and its metabolite cis-2-butene-1,4-dial in two mammalian cell lines at two different loci. Only cis-2-butene-1,4-dial induced a significant increase in mutation frequency at both loci. To gain mechanistic insights into the mutagenic activity of cis-2-butene-1,4-dial the TK mutation assay was exploited by enumerating the small and large mutant colonies. In addition, in the same cell system the induction of micronuclei was investigated in the same mutagenic dose range.

P3: The effects of 2-butene-1,4-dial (BDA), the major metabolite of furan, on primary rat hepatocytes and the rat hepatoma cell line H4IIE were tested.

P4: Changes in the expression of genes involved in DNA damage response were investigated following furan exposure including the dose response. Gene-specific methylation patterns and global DNA methylation were determined following furan treatment using furan induced cholangiocarcinoma samples as a reference.

P5: Completion of cytogenetic profile of furan and its key metabolite cis-2-butene-1,4-dial in vitro in human lymphocytes and two human lymphoblastoid cell lines from group “A” Fanconi’s anemia patients [LFA145(-/-); LFA8(-/-)] and two cell lines from normal individuals [LFA194(+/+); LFA195(+/+)] which served as controls. The use of Fanconi’s anemia cells has been justified by their hypersensitivity to DNA cross-link agents since cis-2-butene-1,4-dial is a DNA cross-linking agent. Furthermore, a panel of DNA-repair-defective Chinese hamster cells which result in a greatly enhanced cytogenetic effects of the compound assayed when a relevant key enzyme in a specific DNA repair pathway is missing or compromised was also employed, e.g. (UV4) defective for nucleotide excision; (EM9) defective for base excision repair; (irs1SF) defective for homologous recombination; (V3) defective for non homologous end-joining recombination (NHEJR).

In vivo, following oral administration of furan in rats for 28 days, evaluation of chromosome aberrations and micronuclei in bone marrow erythrocytes and chromosomal aberrations in resting G_0 splenocytes was performed. Furthermore, DNA damaging activity under the above reported experimental conditions has been evaluated by the alkaline comet assay in blood, bone-marrow and liver cells.

P6: About one hundred samples were collected (fruit and vegetables juices, nutrition drinks and bakery products) and analysed for furan content. The results were combined with those from the preceding years and added to the existing data base on furan levels in food (project deliverable D8.1) to create the project deliverable D8.2. The analytical method was validated and the results were described in a validation report (project deliverable D8.3).

Results achieved

In the first year, the 28-day oral toxicity study only showed minor liver damage induced by furan both in rats and in mice, but analysis of the tissues and blood samples already indicated changes in gene expression and bile acid excretion. In addition, a number of biliary

metabolites formed from furan or its reactive metabolite were characterized. Samples for analysis by accelerator mass spectrometry have been generated and will be analysed in spring 2008. The genotoxicity studies performed showed equivocal results with furan itself but a clear response with the reactive furan metabolite when added directly to the cell culture media. The summary of furan concentrations shows a wide range of encountered furan concentrations in food and identified some further needs to analyse specific food items for furan content. The mechanistic studies have indicated specific pathways of furan formation which require confirmation in further studies.

The performed studies are in line with the workplan of the project and will serve as a basis for further development of mode-of-action-models for furan. The results will provide data on the mode-of-action of furan induced liver carcinogenesis as a basis for a conclusive assessment of health risks in humans due to dietary exposure. Combining these findings will provide a risk/benefit analysis and a scientific basis to justify limits for human furan exposures. All results of the project will be published in peer-reviewed scientific journals to make the scientific and regulatory community aware of the project results and as a measure of project success. Assessment and evaluation of WP results and progress towards the objectives will be monitored by the participants in each workpackage.

In the second year following results were obtained:

The ^{14}C content in DNA extracted from low and high dose animals was significantly increased in a dose dependent manner. While these results indicate that furan may bind to DNA, it is important to exclude the possibility that the increased ^{14}C content in DNA extracted from furan dosed animals is due to metabolic incorporation into DNA. Detailed analysis indicated that radioactivity in DNA did not coelute with normal nucleoside suggesting covalent modification.

No overt signs of liver toxicity were evident by histopathology, clinical chemistry and metabonomics after oral administration of furan for 28 days. However, a dose-dependent increase in cell proliferation was detected specifically in areas located near the edge of the liver lobes, with the caudate lobe being particularly susceptible. Consistent with these changes, treatment with furan led to up-regulation of a number of apoptosis and cell cycle related genes. No global DNA methylation change or gene-specific methylation change were found at the dose levels employed.

In vivo genotoxicity assays in B6C3F1 mice provided evidence of genotoxic effects after repeated oral exposure with furan. In particular, a dose related, statistically significant increase of micronuclei was observed, following in vitro stimulation, in binucleated splenocytes from animals exposed in vivo (4 to 15 mg/kg bw). No similar increase was seen in splenocytes from animals administered with single high furan doses (15 to 250 mg/kg bw) Conversely, in liver a significant induction of DNA damage, as detected by comet assay, was only observed after high dose (250 mg/kg b.w.) acute exposure. Furan treatment was associated with a marginal increase in oxidative DNA damage, limited to some organs (mainly the lung). When oxidative DNA damage associated with furan exposure was investigated in animals defective in the repair of 8-oxoG (the *Ogg1*$^{-/-}$ C57Bl/6 mice) no amplification of the levels of this oxidized purine was observed.

A dose-related increase in hprt mutation frequency was observed after treatment of V79 cells with cis-2-butene-1,4-dial, but not with furan. Furan induced a dose-related increase in tk mutation frequency in L5178Y cells, but at high cytotoxic doses. Furan and its metabolite did not induce directly DNA ssb but produced dose-related increases in 8-oxoG. Up-regulation of 3-methyladenine DNA glycosylase, a marker of inflammation, was detected after exposure to furan.

Findings *on in vitro* cytogenetic analysis of furan and its metabolite in “normal” and Fanconi’s anemia cell lines support for a genotoxic (clastogenic) activity of furan following metabolic conversion through its key metabolite, cis-2-butene-1,4-dial.

Analyses of furan levels in fruit juices confirm the information from the literature (*i.e.*, a wide variation), whereas levels in nutrition drinks are much lower than found in the literature. Levels in bakery products are higher than reported in the literature. There seems to be a correlation between the heat treatment of the carrot juice samples and the furan levels. No correlation between the nutrition drink ingredients and the furan levels could be found, though. Studies performed up to now have brought already a good understanding of the mechanisms of furan formation during heat treatment of food which allows suggesting of several mitigation strategies.

In the third year following results were obtained:

P1: The results obtained demonstrate that only a minor amount of radiocarbon is associated with normal nucleosides, suggesting that the increase in ^{14}C-content in DNA extracted from livers of rats treated with ^{14}C-furan is due to covalent binding.

In summary, chemical characterization of furan metabolites in bile provided further evidence to suggest that GSH-conjugates and degraded protein adducts are major in vivo metabolites of furan, consistent with the hypothesis that cytotoxicity mediated through binding of *cis*-2-butene-1,4-dial to critical target proteins is likely to play a key role in furan toxicity and carcinogenicity. Although no toxicity data are available so far, the metabolites identified in bile are assumed to be less toxic than cis-2-butene-1,4-dial and the initial mono-GSH conjugate. Biliary toxicity of furan may thus not to be a result of exposure of the biliary epithelium to high concentrations of reactive metabolites, but may occur secondary to hepatocyte damage.

P2: In studies in B6C3F1 mice, repeated oral furan administration was shown to induce in vivo primary DNA damage in spleen cells, which evolved in double strand breaks following mitogen stimulation of cells in vitro. Direct evidence of induction of DNA damage in the liver was only obtained following a high dose, acute exposure; upregulation of DNA damage response genes was however observed after repeated oral administration of a lower, non toxic furan dose.

Organs in which oxidative DNA damage accumulates following a 28-day Furan exposure were identified. While no treatment related increases in DNA 8-oxoG were detected in the brain, kidney, small intestine or spleen of wild-type C57BL/6 mice exposed to any Furan dose, 1.9- and 1.7-fold increases in 8-oxoG levels were observed in the lung and in the liver, respectively. Low doses of Furan exposure (8 and 15 mg/kg) were found to be associated with increased proliferation of hepatocytes and Kupffer cells. The highest Furan dose (30mg/kg) led to portal, periportal and intralobular damage, as shown by fatty degeneration, presence of apoptotic cells as well as necrotic hepatocytes. In addition inflammatory cells were found to surround enlarged and fibrotic portal tracts. Our data indicate that increased levels of 8-oxoG are mostly associated with increased cell proliferation in the liver. Furan-induced clastogenic activity was confirmed to be independent from the ability to induce oxidative DNA damage.

The experiments to investigate mutagenesis of Furan and its metabolite in V79 and L5178Y rodent cells at the hprt and TK locus respectively were completed. A significant increase in mutation frequency was only detected in the case of cis-2-butene-1,4-dial in a very narrow range of concentrations in both mutation assays. In the case of the TK assay the enumeration of small and large colonies showed an increase in the relative number of small colonies indicating the occurrence of large deletions/gross rearrangements. This mode of action was also confirmed by the significant increase in micronuclei frequency in the same range of doses where the mutagenic activity was displayed. DNA 8-oxoguanine levels increased significantly upon treatment with either Furan or cis-2-butene-1,4-dial suggesting that DNA oxidation thus not play a major role in the mutagenic activity of cis-2-butene-1,4-dial.

P3: The effects of 2-butene-1,4-dial (BDA), the major metabolite of furan, on primary rat hepatocytes and the rat hepatoma cell line H4IIE were tested. With an EC_{50} of 0.59 mM a three-fold higher sensitivity in H4IIE was determined.

The spontaneous reaction of BDA with glutathione resulted in a multitude of products. As this reaction mixture showed cytotoxic effects on HepG2, the major metabolites were isolated and tested separately for cytotoxicity.

P4: Modulation of DNA damage-related genes and limited DNA-methylation change were found at a relatively high dose (30 mg/kg bw) but not at the lower doses (up to 2 mg/kg bw).

P5: In vivo, following oral administration of furan in rats for 28 days **positive** results obtained for induction of **chromosomal aberrations** and for **DNA breakage** (Comet assay) were observed in G_0 **splenocytes** isolated from spleen of control and furan-treated animals, and stimulated to grow *in vitro* following stimulation with concanavalin A® and in **liver** (*high dose, 4 weeks treatment + 1 weeks recovery*) respectively. **Negative** results were obtained for induction of **micronuclei, SCE's** and **chromosomal aberrations** in **bone-marrow** cells and for DNA breakage in **bone marrow** and **blood** cells. Negative findings obtained for these end-points in the proliferating bone marrow cells could be ascribed to a **limited availability** of furan metabolites in this tissue at dose-levels employed and a **selective elimination** of cells bearing aberrations due to proliferation. Conversely, positive findings observed in splenocytes which are resting in the G_0 phase of cell cycle and therefore exposed to the test agent or its metabolites for the whole length of treatment without any selection of damaged cells as in the case of proliferating cells (e.g. bone-marrow cells) allows accumulation of DNA lesions which are then converted into cytogenetic damage, following DNA repair activities. On the bases of the results obtained **furan** proved to be an ***in vivo*** **genotoxin** in rats under the reported experimental conditions, though in **"non-conventional" genotoxicity** assays.

In vitro, furan did not induce chromosomal aberrations in "normal" human lymphocytes in the presence of S9 metabolism, though treatments were performed at the maximum allowable conditions in vitro (e.g. 3 hours treatment and 10 mM highest dose-level). Conversely, furan induced elevated levels of chromosomal aberrations in Fanconi's anemia cell lines at 10 mM concentration in the presence of S9 metabolism (CYP2E1). Due to the hypersensitivity of Fanconi's anemia cell lines to DNA cross-linking agents, clastogenicity of furan is likely to be caused by its DNA cross-linking key metabolite cis-2-butene-1,4-dial (BDA). Negative results obtained in in human lymphocytes in the presence of S9 metabolism (CYP2E1) following treatment with Furan can be explained by the fact that furan is converted into DNA-reactive metabolites but at a rate not sufficient to be detected by "conventional" genotoxicity assay.
Alternatively, Fanconi's Anemia cells which are defective in the pathway to repair DNA cross-links are sensitive enough to detect furan genotoxic metabolites. In the case of **cis-2-butene-1,4-dial** it was hardly detected as clastogenic compound in "normal" human lymphoblastoid cells only in the long treatment (24 hours) at the highest dose-level selected for scoring (23.2 µM). When using Fanconi's anemia cell lines which are hypersensitive to DNA cross-linking agents, **cis-2-butene-1,4-dial** proved to be highly clastogenic even in the short treatment time (4 hours), thus indicating that **cis-2-butene-1,4-dial is likely** acting as a DNA cross-linking agent. This observation is further supported by results obtained when cell cultures are allowed to repair for 12 hours following 24 hour treatment (36 hr sampling time). The incidence of chromosomal aberrations return to control values in the "normal" lymphoblastoid cell line **LFA195 (+/+)** while in Fanconi's anemia cells elevated levels of chromosomal damage still remains.
Results obtained indicate that furan is a subtle in vitro promutagen whose genotoxicity is likely to be caused by its DNA cross-linking key metabolite cis-2-butene-1,4-dial. It can be detected as in vitro genotoxin in "non conventional" cytogenetic assays due to the limited quantities of active metabolite converted in vitro and its elevated reactivity with key structures of cells (e.g. protein, DNA etc.). Furthermore, due the elevated toxicity of cis-2-butene-1,4-dial it can be detected as genotoxin only in a very narrow range of dose-levels (15 – 50 µM).

This last propertie probably explains the numerous negative findings reported in literature.

P6: Analysis of furan in fruit and vegetables juices showed that elevated furan levels were found in carrot juices with relatively high pH-values and prune juices. Other flavours of the brands that have high furan levels in their carrot or prune juices had low furan levels. Carotene in combination with sterilisation is a possible source of furan in carrot juices. The prune drying process is a possible source of furan in prune juices. Babies may have high exposure to furan when their carrot juice consumption is high (0.25 litre per day). Adults are not at risk when consuming carrot or prune juices.

Only one type of pharmaceutical nutrition drink showed high furan levels, regardless of the flavour. Other types of the same brand and all types of other brands had low furan levels. Patients that exclusively consume the high furan level type at the recommended intake (substitution of all meals by nutrition drinks) have a high exposure to furan. None of the nutrition drinks that are available in the cooled shelves of supermarkets had detectable furan levels. No furan source could be detected in the pharmaceutical nutrition drink. Conservation heat treatment may play a role in furan formation. Heat treatment is applied in pharmaceutical nutrition drinks, not in the short shelf life supermarket products.

Furan determination in bakery products turned out to be irreproducible, possibly because of inhomogeneous furan distribution in the products. The highest furan levels were found in wholegrain products. The baking process is the probable furan cause. Consumers of even the highest furan containing products have no significant furan exposure, though.

The furan method performance was evaluated through a validation process. The method performs well for liquid and viscous products. For solid products, however, the reproducibility is very low, leading to elevated limits of detection and quantification.

Summary of project results

Due to genotoxicity and DNA-binding of furan established in the project, the current risk assessment for furan requires application of a linear dose response relationship. Exposures to furan in adults are estimated to be less than 0.5 µg/kg bw/day, data from this project also support a similar intake of furan in children. Due to the high incidence of tumors induced by furan in rats even at the lowest applied dose, a linear extrapolation of the animal data to calculate possible human tumor risks is uncertain. When using a margin-of-exposure assessment, MoEs to a dose inducing a significant tumor incidence are calculated as only 1000 to 2000. The low MoEs warrant further reductions in furan-exposures.

Dissemination and use

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Conference:				
Sept. 2007	www.china.europa.net Created awareness of contamination in food and opportunities on trade of safe food.	Research/ Industry	China Europe	40	P7
Sept. 2008	EEMS annual meeting, Cavtat, Croatia	Research	Europe	250-300	P2, P5
March 2009	SOT Meeting in Baltimore, www.toxicology.org	Research, Industry, Academia	All	> 1000	P1
Aug. 2009	EMS/ICEM Meeting in Firenze, Italy	Research, Industry, Academia	All	> 1000	P1, P5
Sept. 2009	EUROTOX Meeting in Dresden, Germany	Research, Industry, Academia	All	> 1000	P1, P3, P4, P5
March 2010	SOT Meeting in Salt Lake City	Research, Industry, Academia	All	> 1000	P1
July 2010	IUTOX Meeting in Barcelona, Spain	Research, Industry, Academia	All	> 1000	P1, P4
	Publications:				
2007	Formation of furan and methylfuran from ascorbic acid in model systems and food (published in: *Food Additives and Contaminants*)	Toxicologists			P8
2008	Formation of furan and methylfuran by Maillard-type reactions in model systems and food (published in: *Journal of Agricultural and Food Chemistry*)	Toxicologists			P8
Dec. 2009	Gene expression and DNA methylation changes in rat liver following exposure to furan (in press in: *Toxicological Sciences*)	Research			P4
Nov. 2009	Furan levels in fruit and vegetables juices, nutrition drinks and bakery products (submitted to: *Analytical Chimica Acta*)	Research			P6
Dec. 2009	Hepatobiliary toxicity of furan: Identification of furan metabolites in bile of male F344/N rats (submitted to: *Drug Metabolism and Disposition*)	Research			P1
Jan. 2010	Functional and proliferative effects of repeated low-dose oral administration of furan in rat liver (submitted)	Research			P1
2010	Scientific publication on DNA-binding of furan in rat liver (in preparation)				P1
2010	Scientifc publication on identification of target proteins of furan in rat liver (in preparation)				P1

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Jan. 2010	Assessment of in vivo genotoxicity of the rodent carcinogen furan: evaluation of DNA damage and induction of micronuclei in mouse splenocytes (published in *Mutagenesis*)	Research			P2
Febr. 2010	Evidence of oxidative stress and associated DNA damage, increased proliferative drive, and altered gene expression in rat liver produced by cholangiocarcinomic agent furan (published in: *Toxicol. Pathol.*)	Research			P4
March 2010	Toxic and genotoxic effects of oral administration of furan in mouse liver (published in *Mutagenesis)*	Research			P2
2010	Publication on induction of oxidative DNA damage by furan (in preparation)	Toxicologists			P2
2010	Cytogenetic profile of Furan in mammalian cells in vitro (in preparation for submission to Mutation Research)	Toxicologists			P5
2010	Mechanistic aspects of genotoxicity of furan and its its key metabolite cis-2-butene-1,4-dial in mammalian cells in vitro (in preparation for submission to Mutation Research)	Research			P5
	Oral presentations at international conferences				
Aug. 2009	A. Mally: The Role of Genetic and Non-genetic Mechanisms in Furan Risk (Session: *Prevention of mutation-related diseases* DIETARY FACTORS, MUTATION AND CANCER), EMS/ICEM, Firenze, Italy	Research, Industry, Academia	All	>1000	P1
Aug. 2009	P. Mosesso: Mechanistic Aspects of Genotoxicity of Furan and its Key Metabolite Cis-2-butene-1,4-dial in Mammalian Cells In Vitro (Session: *Prevention of mutation-related diseases* DIETARY FACTORS, MUTATION AND CANCER), EMS/ICEM, Firenze, Italy	Research, Industry, Academia	All	>1000	P5
Sept. 2009	K. Chipman: Role of genotoxic and non-genotoxic mechanisms in furan carcinogenicity (Session: Environmental Chemicals and Food Contaminants: Are They a Risk to Public Health?) EUROTOX Dresden	Research, Industry, Academia	All	> 1000	P4
Sept. 2009	S. Moro: Identification of target proteins of furan in rat liver (EUROTOX, Dreseden)	Research, Industry, Academia	All	> 1000	P1
Sept. 2009	C. Hamberger: Analysis of DNA binding of furan in rat liver by accelerator mass spectrometry (EUROTOX, Dreseden)	Research, Industry, Academia	All	> 1000	P1
Sept. 2009	T. Chen: Modulation of hepatic gene expression and DNA methylation in furan treated Sprague-Dawley Rats (EUROTOX, Dreseden)	Research, Industry, Academia	All	> 1000	P4
Sept. 2009	P. Mosesso: Cytogenetic effects of furan and its key metabolite cis-2-butene-1,4-dial in mammalian cells *in vitro* (EUROTOX, Dreseden)	Research, Industry, Academia	All	> 1000	P5

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Aug. 2010	W. Dekant: Heat-treatment induced toxicant in food: Needs for health risk assessment (Session: Toxicity and risk assessment of heat-treatment formed products)	Research, Industry, Academia	All	> 1000	P1
Aug. 2010	K. Chipman: Role of genetic and epigenetic mechanisms in furan toxicity (Session: Toxicity and risk assessment of heat-treatment formed products)	Research, Industry, Academia	All	> 1000	P4
June 2007	**Project web-site**	http://www.furan-ra.toxi.uni-wuerzburg.de			P1
July 2008	Partner web-site	http://www.ivm.falw.vu.nl/Research_projects/			P6
	Posters				
28. Sept. 2007	Potentielle Gefährdung des Menschen durch Furan; Symposium of "Food Chemistry and Toxicology – Cornerstones of Life Science in Chemistry", Kaiserslautern 2007	General	Germany	100	P3
8. - 10. Sept. 2008	Potentielle Gefährdung des Menschen durch Furan;37. Deutscher Lebensmittelchemikertag; Kaiserslautern 2008	General	Germany	> 300	P3
21 sept. 2008	Assessment of in vivo genotoxicity and cytotoxicity of furan in the mouse. 38th Annual Meting of the European Environmental Mutagen Society. Cavtat, Croatia	General	World Wide	>300	P2
2-5 Nov. 2008	Evaluation of genotoxic activity of hepatic carcinogen Furan in mammalian cells in vivo and in vitro; 6th Pan African Environmental Mutagen Society Conference Cape Town, South Africa,	General	Africa	> 300	P5
15-19 March 2009	Furan in Food: 28 Day Oral Toxicity and Cell Proliferation in Male F344/N Rats Session Title: Safety Issues Concerning Food Products and Micronutrients (SOT Baltimore)	General	World-wide	>1000	P1
15-19 March 2009	Modulation of hepatic gene expression independent of DNA methylation in furan treated F344/N Rats Session Title: Safety Issues Concerning Food Products and Micronutrients (SOT Baltimore)	General	World-wide	>1000	P4
14. Sept. 2009	S. Moro*, C. Hamberger, W. Dekant, J.K. Chipman and A. Mally: Identification of target proteins of furan in rat liver (EUROTOX, Dreseden)				
14. Sept. 2009	C. Hamberger*, S. Moro, M. Malfatti, K. Turteltaub, A. Mally and W. Dekant: Analysis of DNA binding of furan in rat liver by accelerator mass spectrometry (EUROTOX, Dreseden)				
14. Sept. 2009	T. Chen*, K. Hickling, C. Hamberger, A. Mally and J.K. Chipman: Modulation of hepatic gene expression and DNA methylation in furan treated Sprague-Dawley Rats (EUROTOX, Dreseden)				

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
14. Sept. 2009	P. Mosesso*, G. Pepe, R. Bellacima, M. Fiore and S. Cinelli: Cytogenetic effects of furan and its key metabolite cis-2-butene-1,4-dial in mammalian cells *in vitro* (EUROTOX, Dreseden)				
14. Sept. 2009	J. Brück, T. Schneider and D. Schrenk In vitro toxicity of furan and its metabolite(s) in liver cells (EUROTOX, Dreseden)				
4-6 Nov. 2009	Analysis of furan in food; International Symposium on Recent Advances in Food Analysis (RAFA) 2009, Prague	General	World-wide	>300	P6
March 2010	S. Moro, C. Hamberger, W. Dekant, J.K. Chipman and A. Mally: Identification of target proteins of furan in rat liver (SOT, Salt Lake City)	Research, Industry, Academia	World-wide	> 1000	P1
March 2010	C. Hamberger, S. Moro, M. Malfatti, K. Turteltaub, A. Mally and W. Dekant: Analysis of DNA binding of [3,4-^{14}C]-furan in rat liver by accelerator mass spectrometry (SOT, Salt Lake City)	Research, Industry, Academia	World-wide	>1000	P1

Publishable results

Result description (product(s) envisaged, functional description, main advantages, innovations)	1. Data base (web-based report) on existing data on furan in food 2. Analysis of furan in food in various food items 3. Strategies for furan reduction in food systems 4. Risk assessment of furan
Possible market applications (sectors, type of use ..) or how they might be used in further research (including expected timings)	n.a.
Stage of development (laboratory prototype, demonstrator, industrial product...)	n.a
Collaboration sought or offered (manufacturing agreement, financial support or investment, information exchange, training, consultancy, other)	n.a
Collaborator details (type of partner sought and task to be performed)	n.a
Intellectual property rights granted or published	n.a
Contact details	n.a

Results description

1. – 2. Data base (web-based report) on existing data on furan in food and analysis of furan in food in various food items

In the framework the project "Role of genetic and non-genetic mechanisms in furan risk" (Furan-RA), a "Database (web-based report) on furan in food" has been produced. The data base comprises the result of an inventory of data on furan concentration levels in food, as published in the scientific literature and by regional, national and European food safety authorities, as well as the results of the furan in food determinations as performed by the WP

8.1 project partners. The aim of the database is to provide stakeholders with data on furan levels in food.

This report can be downloaded as pdf file or as Excel sheet from the public area of the project home-page located at http://www.furan-ra.toxi.uni-wuerzburg.de/ (Deliverables 8.1, 8.2 and 8.3). Part of the data is taken from publications in the scientific literature, from reports or from websites. The data sources were retrieved by scientific literature searches, internet searches, visits to websites of institutes that were known to have worked on furan determination in food, as well as direct approaches of the latter category institutes. The remaining data were generated in the project itself, by two of the WP 8.1 partners, who have analysed nearly 200 food samples.

3. Strategies for furan reduction in food systems

The formation of furan from linoleic and linolenic acid, respective triglycerides, and (*E,E*)-2,4-decadienal was studied in the presence of various antioxidants, prooxidants, and inert atmosphere. Study was carried out in model systems simulating food processing such as roasting and pressure cooking (sterilization). The formation of furan considerably varied depending on antioxidant, precursor (free PUFA vs. triglyceride) and conditions of heating including pH. Under roasting conditions all precursors except (*E,E*)-2,4-decadienal showed unexpected increase (from +37 to +89 %) of furan formation in the presence of BHT and a-tocopherol. Under pressure cooking conditions, BHT revealed significant effect on furan reductions (from -45 to -100 %) in all systems investigated. Contrariwise, effects of a-tocopherol and ascorbyl palmitate resulted in both decrease and increase of furan formation depending mainly on pH and precursor. When precursors were heated in a nitrogen atmosphere, the furan yields from all precursors were lowered under both roasting (from -87 % to -92 %) and pressure cooking (from -68 % to -98 %) conditions. Under sterilization conditions (pH 4 and 7), ascorbic acid revealed a strong prooxidative effect on linoleic acid leading to a growth of furan formation. The effect was higher at pH 7 than at pH 4 and increased with concentration of ascorbic acid. The generation of furan from ascorbic acid alone was minor compared to PUFA precursors.

It was observed that effects of antioxidants on furan formation considerably varied depending on antioxidant, precursor (free PUFA vs. triglyceride) and conditions of heating including pH. Fortification of foods by a-tocopherol prior to heating seems to be a promising mitigation strategy, which should be further investigated. Furthermore, it is well known that tocopherols posses a strong synergistic effect with AA, which could allow to develop a very efficient antioxidant system for reduction of furan formation. Nevertheless the concentration of AA should be properly optimized with regard to simultaneous prooxidant properties. Modification of atmospheres within heating systems seems to be also very promising mitigation concept. However, such a strategy would require a significant modification of technological process. Obtained data confirmed that AA in the mixture with PUFA acts as a prooxidant rather than direct furan precursor. This effect is higher at pH 7 than at pH 4 and increases with concentration of ascorbic acid. Surprisingly, no suppression of furan generation was observed as one would expect due to known antioxidant effect of AA at high levels. The results showed that effect of AA is not linked with decrease of pH after its addition as speculated before.

4. Risk assessment of furan

Due to genotoxicity and DNA-binding of furan established in the project, the current risk assessment for furan requires application of a linear dose response relationship. Exposures to furan in adults are estimated to be less than 0.5 µg/kg bw/day, data from this project also support a similar intake of furan in children. Due to the high incidence of tumors induced by furan in rats even at the lowest applied dose, a linear extrapolation of the animal data to

calculate possible human tumor risks is uncertain. When using a margin-of-exposure assessment, MoEs to a dose inducing a significant tumor incidence are calculated as only 1000 to 2000. The low MoEs warrant further reductions in furan-exposures. A detailed report can be downloaded as pdf file from the public area of the project home-page located at http://www.furan-ra.toxi.uni-wuerzburg.de/ (Deliverable 9.1).