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FURAN
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FURAN

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1. EXPLANATION

Furan (C_4H_4O) (Chemical Abstracts Service [CAS] No. 110-00-9) is a highly volatile cyclic ether that can be formed unintentionally in foods during processing from precursors that are natural food components. Information available to the Committee at its present meeting suggested that the major route of exposure to

furan in the human population is through consumption of heat-treated foods and beverages.

Furan has not been evaluated previously by the Committee. The request for a full evaluation of furan originated from the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

Searches for relevant publications were performed utilizing various search engines and databases, including, but not limited to, RefWorks, PubMed, Google, SpringerLink.com, Wiley Interscience and Elsevier. Methods involved keyword searches, journals, article titles, authors and previously cited references in related material.

2.1.1 Absorption, distribution and excretion

The disposition of [^{14}C]furan has been studied in male F344 rats. Furan (>99% pure) in corn oil was administered by the intragastric route at a dose of 8 mg/kg body weight (bw) once per day for between 1 and 8 consecutive days. This corresponded to the highest dose tested in the F344 rat in the United States National Toxicology Program (NTP) 2-year assay described below in section 2.2.3. Following dosing, animals were placed in metabolism cages for the separate collection of urine and faeces, as well as the trapping of carbon dioxide and volatiles (animals receiving a single dose only). Animals were sacrificed at time points ranging from 1 to 8 days after dosing, and each of the major tissues was removed for analysis of radioactivity and covalent binding to liver macromolecules.

In the first 24 h after dosing, expired air, urine and faeces were all significant routes of elimination of furan-derived radioactivity. Approximately 14% of the administered dose was expired as unchanged furan, most (11%) within the first hour after dosing. In addition, 26% of the administered dose was expired as carbon dioxide, virtually all of which was eliminated in the first 12 h. Radioactivity in urine and faeces accounted for about 20% and 22% of the administered dose, respectively. By 24 h, 19% of the administered radioactivity remained in the tissues, suggesting that 81% had been eliminated in expired air, urine and faeces. The highest concentration of radioactivity was found in the liver (13% of the dose); the next highest concentrations were found in the kidney, blood and small and large intestines, each accounting for less than 1% of the administered dose (0.45%, 0.42%, 0.15% and 0.13%, respectively). The concentrations in lung tissue were very low, accounting for only 0.02% of the administered dose. Elimination of radioactivity from the liver appeared to follow first-order kinetics, with a half-life of 1.8 days. In contrast, the elimination kinetics for kidney and blood were more complex, with the blood concentration of radioactivity remaining mostly constant for 8 days following a single dose of 8 mg/kg bw. The concentrations of furan-derived radioactivity were higher in the liver, kidney and blood with multiple doses of furan compared with a

single dose by about 6-fold in kidney and blood and 4-fold in the liver. The percentage of administered radioactivity eliminated in the faeces was the same for eight daily doses as for a single dose followed for 8 days. Elimination of radioactivity in the urine, in contrast, increased as a percentage of the total administered dose from 20% of a single dose to 33% of 8 days' cumulative dose.

At 24 h, high-performance liquid chromatographic (HPLC) analysis of plasma or an extract of liver showed no unchanged furan. Approximately 80% of the radioactivity retained in the liver was not extractable by organic solvents; the remaining radioactivity was assumed to be covalently bound to tissue macromolecules. With the techniques available at the time, the authors concluded that this radioactivity was associated with protein and that either there was no binding to deoxyribonucleic acid (DNA) or furan–DNA adducts were not stable to the isolation procedure. Repeated daily administration of [^{14}C]furan resulted in an increase in covalent binding over 4 days, at which point the amount of non-extractable radioactivity reached a plateau. At least 10 metabolites of furan were isolated from the urine, but were not identified. The authors noted that the large amount of administered radioactivity expired as carbon dioxide (26%) indicated that a considerable portion of furan metabolism involves ring opening and oxidation to carbon dioxide. The ring-opened product was predicted to be *cis*-2-butene-1,4-dial (BDA, also known as maleic anhydride), with subsequent hydration to maleic acid and rapid excretion as carbon dioxide in rats. The generation of this citric acid intermediate was considered to be a possible explanation for some of the tissue non-extractable radioactivity in liver protein (Burka, Washburn & Irwin, 1991).

A physiologically based pharmacokinetic (PBPK) model was developed for furan in rats. Model simulations of furan metabolism in the rat, following an oral dose of 8 mg/kg bw, predicted metabolism of 84% of the dose and 16% exhaled as the parent compound (Kedderis et al., 1993). Furan biotransformation kinetics determined with freshly isolated rat hepatocytes in vitro were found to accurately predict furan pharmacokinetics in vivo, suggesting that furan biotransformation kinetics determined with freshly isolated mouse or human hepatocytes could be used to develop species-specific pharmacokinetic models.

The PBPK models were used to compare the bioactivation and liver dosimetry of furan between rodents and humans by the inhalation route. The absorbed dose of furan was approximately 3.5-fold and 10-fold greater in rats and mice, respectively, than in humans following the same inhalation exposure. Similar species differences were observed for the integrated exposure of the liver to BDA, the toxic metabolite of furan, over the same inhalation exposure. Comparison of the initial rate of furan metabolism and the rate of liver perfusion indicated that for each of the three species, furan oxidation occurred at rates that were approximately 13- to 37-fold higher than the rate of furan delivery to the liver via blood flow. This suggested that hepatic uptake and clearance and the hepatic concentration of the toxic metabolite of furan as a function of furan exposure concentration would be limited by hepatic blood flow. As a consequence, the authors concluded that the toxic metabolite formed in the liver would be unaffected by increases in the maximum rate (V_{max}) due to the induction of cytochrome P450 (CYP) 2E1 and that interindividual variations observed in CYP2E1 activity in human populations would

not be expected to have a significant effect on the extent of furan bioactivation in humans (Kedderis & Held, 1996). The hepatic blood flow limitation of biotransformation has also been observed after oral bolus dosing of rapidly metabolized compounds, such as ethanol (Kedderis, 1997). Hepatic CYP2E1 activity may be an important variable in determining the extent of any first-pass metabolism within an individual and therefore the extent of pre-systemic bioactivation following oral exposure. Hepatic first-pass metabolism could also determine the extent of systemic exposure and subsequent elimination in expired air. The absolute bioavailability of orally administered furan has not been studied.

2.1.2 Biotransformation

The finding that furan biotransformation kinetics determined with freshly isolated rat hepatocytes in vitro accurately predicted furan pharmacokinetics in vivo after inhalation exposure was used to develop a hepatocyte suspension/culture system with freshly isolated hepatocytes that utilized furan concentrations and incubation times similar to hepatic dosimetry in vivo. The biotransformation kinetics of furan by freshly isolated male F344 rat hepatocytes in suspension were determined in sealed flasks by measurement of changes in the furan headspace (HS) concentrations as a function of incubation time. The CYP inhibitor, 1-phenylimidazole, and the substrate, ethanol, were potent inhibitors of furan biotransformation. Furan biotransformation was also inhibited by pretreatment of the rats with aminobenzotriazole (ABT), a selective suicide inactivator of CYP. Pretreatment of the rats with phenobarbital to induce the CYP2B family had no effect on the rate of furan oxidation, whereas pretreatment with acetone to induce CYP2E1 increased the initial rate of furan oxidation approximately 5-fold. These results suggested that CYP2E1 is a major catalyst for furan oxidation (Kedderis et al., 1993).

Hepatocytes from male B6C3F1 mice and from three human donors (two males and one female) were also studied in the above suspension culture system. Hepatocytes from mice oxidized furan at a greater rate than found for rats in a previous study (Kedderis et al., 1993) or human hepatocytes. Hepatocytes from the three human donors oxidized furan at rates equal to or greater than those of rat hepatocytes. A greater than 2-fold variation in V_{max} was observed among the three preparations of human hepatocytes. The observation that two of the donors had died in automobile accidents following alcohol consumption was consistent with the interpretation that the higher V_{max} values in these samples relative to the other sample were due to the induction of CYP2E1 by ethanol (Kedderis & Held, 1996).

By analogy with the unsaturated aldehydes that have been identified as the microsomal metabolites of 2-methylfuran and 3-methylfuran (Ravindranath, Burka & Boyd, 1984), BDA was proposed as the reactive microsomal metabolite of furan. Liver microsomal fractions obtained from male F344 rats were incubated with concentrations of furan ranging from 0.025 to 20 mmol/l in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and semicarbazide. Given the reactive nature of the proposed metabolite, the assay required the presence of a trapping agent such as semicarbazide to prevent protein binding. HPLC analysis of the incubation mixtures revealed the formation of a metabolite that co-eluted with standards for the bis-semicarbazone adduct of BDA (Chen, Hecht & Peterson, 1995).

Incubation of furan (2 mmol/l) with F344 rat liver microsomes in the presence of cofactors and [glycine-2-^{3}H]glutathione (GSH) at 4 mmol/l led to the formation of radioactive peaks that co-eluted with synthetic standards for the bis-GSH conjugates (Chen, Hecht & Peterson, 1997). Using an HPLC with electron capture method for detection of GSH conjugates, the formation of bis-GSH conjugates in rat and human microsomes incubated with furan was confirmed, together with a previously uncharacterized type of reaction product, the mono-GSH conjugates (Peterson et al., 2005).

The urinary metabolites of furan from male F344 rats dosed intragastrically with 8 mg/kg bw of either [$^{12}C_4$]furan or [$^{13}C_4$]furan in corn oil were determined. Several metabolites resulting from bis- and mono-GSH conjugates of BDA were predicted, but the only metabolite detected in the 24 h urine was the mono-GSH conjugate. The finding of this metabolite in the urine of furan-dosed rats confirms that BDA is formed and conjugated with GSH in vivo. Although bis-GSH conjugates or their predicted metabolites were not detected in urine, the authors indicated that they might be formed and preferentially excreted via the bile and therefore might be detected in faeces. At least 18 other furan-derived urinary metabolites were detected; preliminary data indicated that they were conjugates and that the furan portion of the molecule derived from further metabolism of BDA (Peterson et al., 2006).

Metabolic profiling of products that had been excreted in the urine of rats during two 24 h periods after intragastric dosing with furan at 40 mg/kg bw in corn oil was conducted using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Seven peaks that increased significantly following dosing were investigated as biomarkers of exposure to furan; the structures of five metabolites were elucidated. Of these, two were identified as being mercapturic acid derivatives (GSH/cysteine conjugates) of furan, one as a lysine adduct and two combining elements of both mercapturic acid derivatives and lysine adducts. While the two exclusively mercapturic acid derivatives were postulated to result from the reaction of BDA with GSH, the three metabolites containing lysine adducts were postulated to be derived from degraded protein adducts (Kellert et al., 2008a).

Further characterization of furan metabolites in urine of male F344 rats dosed intragastrically with 8 or 40 mg/kg bw [$^{12}C_4$]furan or [$^{13}C_4$]furan in corn oil and in freshly isolated hepatocytes from Sprague-Dawley or F344 rats incubated with furan at 100 µmol/l was conducted using LC-MS/MS analysis. It was concluded that the reaction of BDA with GSH does not completely deactivate BDA, as had been suggested previously (Chen, Hecht & Peterson, 1997). Chemical characterization of the urinary and hepatocyte metabolites of furan suggested that there are at least three different types of protein adducts generated by reactive furan metabolites: 1) BDA can react directly with protein lysine residues to generate pyrrolinone adducts; 2) BDA can react with protein thiol groups to generate a protein–thiol BDA intermediate that can then react with protein lysine groups to form a cysteine–BDA–lysine crosslink within a protein; and 3) a GSH–BDA–protein lysine crosslink can be formed (Lu et al., 2009).

In addition to the two urinary metabolites described by Lu et al. (2009), seven additional urinary metabolites have been characterized. All nine metabolites are derived from a common intermediate that results from the crosslinking of BDA to cysteine and lysine residues. Some of these metabolites are among the most abundant furan metabolites in urine, as judged by LC-MS/MS analysis. The authors concluded that the formation of cysteine–BDA–lysine crosslinks is an important component in the overall metabolism of furan in rats and indicated the strong possibility that these urinary metabolites are degraded protein adducts (Lu & Peterson, 2010).

2.1.3 Effects on enzymes and other biochemical parameters

A series of studies was carried out to investigate the covalent binding of furan with hepatic microsomal protein, to identify the CYP enzymes involved in metabolic activation and to determine possible mechanisms by which furan can interact with CYP. ^{14}C-labelled furan was found to be covalently bound to microsomal proteins following incubation with liver microsomes isolated from male F344 rats in the presence of NADPH. Microsomes isolated from phenobarbital- (PB), imidazole- or pyrazole (PY)-treated rats exhibited significantly enhanced covalent binding of furan to protein, indicating the increased formation of reactive species after these pretreatments. However, binding was reduced to almost half of control values when liver microsomes from β-naphthoflavone (β-NF)-treated animals were used. The greater degree of induction observed with imidazole and PY suggests that this metabolism-dependent covalent binding is preferentially catalysed by the CYP2E1 enzyme. To determine the nature of the functional groups involved in covalent binding of furan to microsomal proteins, binding was studied in the presence of compounds acting as nucleophiles: containing amine groups available for binding (semicarbazide); containing an amine group blocked for binding and a thiol group available for binding (*N*-acetylcysteine [NAC]); and containing both amine and thiol groups available for binding. GSH, with both free amine and thiol groups, provided maximum protection against binding for microsomes from control, PB-, imidazole- and β-NF-treated rats, reducing binding in control microsomes by 86%. Semicarbazide also provided significant protection against covalent binding (control, PB- and imidazole-treated), whereas NAC provided protection only in microsomes from imidazole-treated rats. Incubation of microsomes from rats dosed intragastrically with furan in corn oil at 0, 8 or 25 mg/kg bw resulted in a dose-related decrease in CYP concentration and in decreased activities of aniline hydroxylase (AH), ethoxyresorufin-*O*-deethylase (EROD) and 7-ethoxycoumarin-*O*-deethylase (ECOD) after 24 h, suggesting that the reactive species produced during CYP-catalysed metabolism reacts with nucleophilic groups located within the CYP itself. No significant inhibition of EROD or ECOD activity or significant change in the carbon monoxide binding spectrum of the CYP or in total haem content was noted in microsomes from untreated rats that were incubated for 5, 10 or 25 min in either the presence or absence of NADPH; an inhibition of AH activity (30%) was noted after incubation with furan for 5 min, with or without NADPH. When microsomes from rats treated with PB or PY were incubated with furan, no significant decrease in CYP concentration or in total haem content was noted compared with microsomes from untreated animals. In microsomes from PB-treated rats incubated with furan,

a significant increase in the activity of ECOD was noted, whereas the activity of EROD was unaffected and that of AH was significantly decreased. Incubation of microsomes from PY-treated rats with furan reduced the activities of EROD and AH, but did not affect the activity of ECOD, compared with controls. Studies conducted to investigate the covalent interaction with the haem and protein moieties of CYP found that furan-derived radioactivity was distributed almost equally to the haem and protein moieties. The authors were not able to characterize the chemical nature of the haem–furan adduct (Parmar & Burka, 1993).

An isolated male Fischer rat hepatocyte suspension system was used to show that furan-induced cytolethality can be produced in vitro via an apparent solvent effect at high (millimole per litre) concentrations or via a reactive metabolite at concentrations (micromole per litre) relevant to tissue doses in vivo. At conditions that more accurately reflect the dosimetry of hepatotoxic doses of furan in vivo, furan (2–100 µmol/l) produced a concentration- and time-dependent decrease in GSH concentrations and cell viability of hepatocytes. Addition of the CYP inhibitor, 1-phenylimidazole, prevented the furan-induced depletion of GSH and cytolethality. In contrast, pretreatment of the rats with the CYP2E1 inducer, acetone, increased GSH depletion and cytolethality at a concentration of 100 µmol/l, but not at 2 or 10 µmol/l. These data indicated that the oxidation of furan by CYP is required for GSH depletion and cytolethality in vitro at furan concentrations that are relevant in vivo (Carfagna, Held & Kedderis, 1993).

Incubation of isolated rat hepatocytes at concentrations (2–100 µmol/l) that had been found by Carfagna, Held & Kedderis (1993) to be relevant to in vivo exposure produced irreversible concentration- and time-dependent reductions in adenosine triphosphate (ATP) concentration that preceded cell death. In light of the fact that mitochondria produce about 95% of the total ATP needs of eukaryotic cells, the effect of furan on mitochondrial respiration in rat hepatocytes both in vivo and in vitro was investigated. Furan doses of 15 or 30 mg/kg bw administered intragastrically in corn oil to male F344 rats were found to greatly increase state 4 (succinate-stimulated) respiration in mitochondria of hepatocytes in a dose-related manner, without affecting state 3 (adenosine diphosphate [ADP]–stimulated) respiration. In addition, these doses of furan increased the activity of mitochondrial adenosine triphosphatase (ATPase) in hepatocytes of treated rats. Stimulation of oxygen uptake by mitochrondria in the absence of ADP and increased activity of the ATP-hydrolysing activity in mitochondria are characteristic of irreversible uncouplers of mitochondrial respiration. Similar results were found with the in vitro hepatocyte model. Pretreatment of rats or co-incubation of hepatocytes with 1-phenylimidazole prevented the effects of furan on mitochondrial respiration, indicating that biotransformation of furan was required for its cytotoxic activity. The authors concluded that furan-induced uncoupling of oxidative phosphorylation is an early, critical event in cytolethality both in vivo and in vitro (Mugford, Carfagna & Kedderis, 1997).

Blood was collected from F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day) in the Gill et al. (2009) 90-day intragastric study (see section 2.2.2) for analysis of oxidative stress and antioxidant defences. Furan did not cause significant changes in circulating 8-hydroxy-2′-deoxyguanosine, total

antioxidant capacity or oxidized low-density lipoprotein. Paraoxanase-1 was increased at 2 mg/kg bw in males and at 8 mg/kg bw in females (Jin, Kearns & Coughlan, 2009).

2.2 Toxicological studies

Searches for relevant publications were performed utilizing various search engines and databases, including, but not limited to, RefWorks, PubMed, Google, SpringerLink.com, Wiley Interscience and Elsevier. Methods involved keyword searches, journals, article titles, authors and previously cited references in related material.

2.2.1 Acute toxicity

(a) Mice

A single intraperitoneal dose of 300 mg/kg bw of furan in sesame oil was administered to 10 adult male Swiss albino mice; in addition, a group of 25 mice received piperonyl butoxide, an inhibitor of CYP, at 1360 mg/kg bw intraperitoneally 30 min prior to injection with furan. Control mice (number not indicated) received the vehicle. The animals were sacrificed 36 h after being dosed with furan. The livers and kidneys were removed and samples prepared for histopathological evaluation. At 300 mg/kg bw, furan produced a moderate to severe coagulative necrosis in both organs. In the liver, the lesions were concentrated in the centrilobular zone. Coagulative lesions of the kidney appeared to affect the proximal convoluted tubules of the outer cortex, without damaging glomerular or medullary cells. Very little tubular precipitate or tubular dilatation was present. Pretreatment with piperonyl butoxide resulted in eradication of kidney lesions and a reduction of severity of the liver lesions to mainly mild in nature (McMurtry & Mitchell, 1977).

The toxicity of single intraperitoneal doses of furan was assessed as part of a study of the hepatic and renal toxicity of a homologous series of 2- and 3-alkylfurans. Male ICR mice, 20–25 g, were initially administered a dose of 2.6 mmol/kg bw (180 mg/kg bw as furan) of each of the furans in sesame oil by intraperitoneal injection. Plasma urea nitrogen and plasma glutamate–pyruvate transaminase (GPT) (also known as alanine aminotransferase [ALT]) activity were determined 24 h later to assess the relative potency of renal and hepatic toxicity, respectively, for the compounds. This dose had been previously determined to be the maximum amount of the most potent member of the series that could be given without producing mortality during the course of the experiments. Furan dosing produced a non-statistically significant increase in plasma urea nitrogen that was about 2-fold greater than values for the controls. Of all the compounds tested, furan was the most potent in increasing serum GPT activity over a 24 h period, elevating activity by more than 2 orders of magnitude over control values. The same dose of furan (180 mg/kg bw) had no effect on urine-concentrating ability, compared with controls, in mice that had been deprived of food and water for 48 h. Histopathological evaluation of liver and kidney samples taken 24 h after a single intraperitoneal furan dose of 5.1 mmol/kg bw (350 mg/kg bw) revealed massive centrilobular necrosis and slight proximal tubular necrosis, respectively. HPLC analysis of heptane

extracts of liver and kidney tissue at 1, 2 and 5 h after administration of the test compound at 4.1 mmol/kg bw (280 mg/kg bw as furan) demonstrated that, in contrast to the other compounds shown to be hepatotoxic, unchanged furan was not found in high concentration in liver tissue over a 5 h period (Wiley et al., 1984).

The effects of a single intraperitoneal dose of furan (along with a series of 3-alkylfurans and 2-ethylfuran) in sesame oil on lung toxicity were assessed in male ICR mice. Twenty-four hours after mice were administered a furan dose of 5.15 mmol/kg bw, severe bronchiolar necrosis was observed in the lungs of the mice. Thymidine incorporation into the DNA of homogenized lungs of mice dosed intraperitoneally with furan at 1.56 mmol/kg bw (106 mg/kg bw) at 3 days after dosing was found to be 4 times higher in furan-dosed mice than in vehicle-treated controls. HPLC analysis of heptane extracts of lung tissue at 1, 2 and 5 h after test compound administration demonstrated that, in contrast to the other compounds, unchanged furan was not retained in lung tissue over a 5 h period (Gammal et al., 1984).

Male B6C3F1/CrlBR mice (five per group) were administered a single intragastric furan (>99% pure) dose of 50 mg/kg bw in corn oil. This dose was determined to be toxic to the liver without killing the animals during the period of the experiment. Two hours prior to sacrifice, the animals were injected intraperitoneally with methyl-[^{3}H]thymidine for incorporation into replicating cells. A blood sample was taken just prior to sacrifice for the determination of aspartate aminotransferase (AST), ALT and lactate dehydrogenase (LDH). The mice were sacrificed at 12 h, 24 h, 48 h, 4 days or 8 days following dosing with furan; corn oil–administered control animals were sacrificed 48 h after treatment. The livers were removed and prepared for histopathological evaluation in sections stained with haematoxylin and eosin (H&E) and determination of the labelling index (LI) by autoradiographic measurement of thymidine-labelled cells. A dose–response experiment for plasma enzymes was also conducted in which male mice were given a single gavage administration of furan at 0, 15, 28, 37 or 50 mg/kg bw in corn oil. Plasma samples were collected from all dose groups just prior to sacrifice, 24 h after dosing, and the activities of AST, ALT and LDH were determined.

Histopathological evaluation revealed moderate midzonal degeneration and necrosis as early as the first scheduled sacrifice after furan dosing (12 h), peaking at 24 h. Parenchymal cells typically had enlarged, pale-staining cytoplasms and condensed chromatin in the nuclei. Hepatocytes in the vicinity of the central vein were very eosinophilic, whereas those near the portal vein were vacuolated in some, but not all, livers. The subcapsular parenchyma of the visceral surface of the left and caudate hepatic lobes had necrosis with some inflammatory cell infiltrate, primarily at 12 h post-treatment. This was taken to suggest that some of the furan diffused through the stomach wall and into the liver subsequent to intragastric administration. By 48 h, there was extensive cell proliferation observed as cells in S-phase and by mitotic figures. Little or no necrosis was apparent at 48 h. Midzonal inflammation was observed in livers from mice sacrificed at either 48 h or 4 days following furan dosing, primarily in the form of infiltrating neutrophils and macrophages. By 8 days, livers had returned to near normal. A spike in LI of hepatocyte nuclei was noted 48 h after dosing, whereas the LI at other time points was not

significantly elevated above control values. Further evidence of the cytotoxicity of furan was large increases in AST, ALT and LDH that occurred as early as 12 h, reaching a peak at 24 h and declining at subsequent time points to reach normal values by 4 days. The dose–response study conducted for plasma enzymes revealed that at the lowest dose of furan tested (15 mg/kg bw), enzyme activity levels at 24 h were not significantly elevated from control values, but they increased markedly with the higher dose levels (Wilson et al., 1992).

(b) Rats

Male F344 rats/CrlBR rats (five per group) were administered a single intragastric dose of furan (>99% pure) at 30 mg/kg bw in corn oil. This dose was determined to be toxic to the liver without killing the animals during the period of the experiment. Two hours prior to sacrifice, the animals were injected intraperitoneally with methyl-[^{3}H]thymidine for incorporation into proliferating cells. A blood sample was taken just prior to sacrifice for the determination of AST, ALT and LDH. The rats were sacrificed at 12 h, 24 h, 48 h, 4 days or 8 days following dosing with furan; corn oil–administered control animals were sacrificed 48 h after dosing. The livers were removed and prepared for histopathological evaluation of H&E-stained sections and autoradiographic measurement of thymidine-labelled cells. A dose–response experiment for plasma enzymes was also conducted in which male rats were administered a single intragastric dose of furan in corn oil at 0, 8, 15, 22 or 30 mg/kg bw. Plasma samples were collected from all dose groups just prior to sacrifice, 24 h after dosing, and the plasma activities of AST, ALT and LDH were determined.

Histopathological evaluation revealed moderate midzonal degeneration and necrosis in rats receiving the single dose of furan as early as the first scheduled sacrifice after furan treatment (12 h). In rare cases, slight midzonal necrosis was observed that was most pronounced 24 h after treatment. Subcapsular necrosis and inflammatory cell infiltration were seen at 12 and 24 h and were more severe than those observed in the mouse. Necrosis began to subside by 48 h. Inflammation was observed at 24 and 48 h, with foci of inflammatory cell infiltrates still noted at 4 days. The livers had returned to near normal by 8 days. A spike in LI of hepatocyte nuclei was noted 48 h after a single furan dose of 30 mg/kg bw. Plasma enzymes were elevated at both 12 and 24 h following treatment, indicating marked cytotoxicity, returning to near-normal values after 4 days. In the dose–response study, plasma enzyme activities were not elevated above control values at the lowest dose (8 mg/kg bw), but they increased markedly with the higher dose levels (Wilson et al., 1992).

2.2.2 Short-term studies of toxicity

(a) Mice

Groups of six male B6C3F1 mice were administered furan intragastrically in corn oil at 15 mg/kg bw per day, 5 days/week, for 6 weeks. Six days prior to sacrifice, pumps containing [^{3}H]thymidine were implanted subcutaneously for incorporation of thymidine into replicating cells. Sections of liver were taken for histopathology

and autoradiographic measurement of thymidine-labelled cells. At the 1-week sacrifice, only an occasional necrotic cell or small cluster of inflammatory cells was noted. In some animals, the visceral surface of the liver was irregular with small foci of necrotic cells and early fibrosis. At the 3-week sacrifice, the subcapsular parenchyma of the visceral surface had necrosis and inflammation that were more extensive than at week 1. The liver was otherwise normal except for some perinuclear vacuolization of the cytoplasm. At the 6-week sacrifice, a centrilobular pattern of hepatocyte vacuolization was evident. No bile duct proliferation was noted. The hepatocyte LIs for furan-dosed mice were 25.1, 12.0 and 3.2 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.41 and 0.89 for weeks 1 and 6, respectively (Wilson et al., 1992).

Groups of five male and five female B6C3F1 mice (51 days old) were dosed intragastrically with furan (>99% pure) in corn oil at 0, 10, 20, 40, 80 or 160 mg/kg bw per day for 12 days (days 1 through 5, 8 through 12 and at least 2 consecutive dosing days before terminal sacrifice). The mice were weighed at the start of the study and at days 8 and 16. Clinical observations were made twice daily. Complete necropsies were performed on all animals. Three of five male mice receiving 40 mg/kg bw per day and all male mice receiving 80 or 160 mg/kg bw per day died by day 6; four of five females that received 80 mg/kg bw per day and all females that received 160 mg/kg bw per day died by day 6. The body weights of male mice receiving 10 or 20 mg/kg bw per day were significantly increased compared with those of controls; in females from the 10, 20 and 40 mg/kg bw per day groups, body weights were similar to those of controls. Inactivity was the most commonly observed clinical finding in the treated mice. No observations at necropsy were clearly associated with furan treatment (NTP, 1993).

Groups of 10 male and 10 female B6C3F1 mice were dosed intragastrically with furan (>99% pure) in corn oil on 5 days/week for 13 weeks, starting when they were 51 days old. Males were dosed with 0, 2, 4, 8, 15 or 30 mg/kg bw per day; females were dosed with 0, 4, 8, 15, 30 or 60 mg/kg bw per day. The mice were observed twice daily, and clinical observations were recorded weekly. Body weights were taken at the start of the study and subsequently at weekly intervals. Surviving mice were killed at the end of the 13-week treatment period, and necropsies were performed on all animals. Weights of brain, heart, right kidney, liver, lungs and thymus were taken at necropsy. Complete histopathological examinations were made of all mice in the control and high-dose groups. Livers from all groups, with the exception of the lowest dose groups for each species (2 mg/kg bw per day and 4 mg/kg bw per day in rats and mice, respectively), were subjected to histopathological examination. Dosing had no effect on survival of the mice. The final mean body weights of male mice receiving 30 mg/kg bw per day were lower than those of controls; the final mean body weights of male mice in the other dose groups and of all dosed groups of female mice were similar to those of control mice. Clinical observations related to dosing with furan were observed only in the highest-dose females and consisted of reduced activity or inactivity. The absolute and relative liver weights at the two top dose levels (15 and 30 mg/kg bw per day in males and 30 and 60 mg/kg bw per day in females) were statistically increased compared with those of control mice; no other organ weight changes related to dosing were found.

A low incidence of liver lesions was noted, starting at a dose of 8 mg/kg bw per day in male mice and 15 mg/kg bw per day in female mice and increasing with dose, affecting nearly all the animals at doses of 30 and 60 mg/kg bw per day for most lesions (Table 1). The lesions included cytomegaly, degeneration and necrosis of hepatocytes, greenish-yellow to brown pigmentation of Kupffer cells (presumably bile), bile duct hyperplasia (most marked in highest-dose females) and cholangiofibrosis (females only). Hepatocyte lesions usually involved periportal hepatocytes, but extended to other areas of the lobules in more severe cases. Hepatocyte degeneration consisted of varying degrees of clear cytoplasmic vacuolization (NTP, 1993).

(b) Rats

Groups of six male and six female F344 rats were administered furan intragastrically in corn oil at 8 mg/kg bw per day, 5 days/week, for 6 weeks. Six days prior to sacrifice, pumps containing [^{3}H]thymidine were implanted subcutaneously for incorporation of thymidine into replicating cells. Sections of liver were taken for histopathology and autoradiographic measurement of the thymidine LI. Subcapsular focal areas of inflammatory cell infiltrate were observed on the visceral surface at the 1-week sacrifice. Several small foci of inflammatory cells were found scattered randomly in the hepatic parenchyma. Centrilobular hepatocytes were vacuolated, whereas the periportal cells were hypereosinophilic. At the 3- and 6-week sacrifices, a prominent pattern of visceral surface lesions and hypereosinophilic periportal hepatocytes was evident. Bile duct hyperplasia with attendant peribiliary fibrosis was observed in the liver parenchyma. In some cases, inflammatory cells from the subcapsular visceral surface lesion extended into the parenchyma, where they surrounded a small number of degenerated and/or necrotic hepatocytes. The subcapsular inflammation was often associated with a light brown pigment in macrophages within the inflammatory zone. Mild to moderate bile duct proliferation within this zone was evident in some sections. By 6 weeks of furan dosing, livers displayed cholangiofibrosis in subcapsular areas of the left or caudate lobes. Signs of metaplasia localized within these cholangiofibrotic areas were observed in two rats as ducts composed of columnar cells with basal nuclei. These cells resembled intestinal cells and have been referred to variously as intestinal cell metaplasia, bile duct hyperplasia and cholangiofibrosis. Evaluation of these ducts in liver sections that had been processed for autoradiography revealed that the nuclei of the cells that composed the ducts were often incorporating thymidine and were therefore actively proliferating. The hepatocyte LIs for furan-dosed male rats were 3.2, 9.2 and 6.5 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.08 and 0.29 for weeks 1 and 6, respectively. The LIs for furan-dosed females were 11.7, 9.2 and 14.4 for weeks 1, 3 and 6, respectively, compared with LIs for controls of 0.77 and 0.75 for weeks 1 and 6, respectively (Wilson et al., 1992).

Table 1. Incidences of selected non-neoplastic hepatic lesions in mice in the 13-week NTP (1993) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw
Biliary tract												
Cholangiofibrosis	—	—	—	—	—	—	0/10	—	0/10	0/10	4/10*	10/10**
Hyperplasia	0/10	—	0/10	0/10	0/10	2/10	0/10	—	0/10	0/10	8/10**	10/10**
Hepatocytes												
Cytomegaly	0/10	—	0/10	0/10	0/10	10/10**	0/10	—	0/10	0/10	10/10**	10/10**
Degeneration	0/10	—	0/10	0/10	1/10	10/10**	0/10	—	0/10	3/10	10/10**	10/10**
Necrosis	0/10	—	0/10	1/10	1/10	8/10**	0/10	—	0/10	0/10	9/10**	10/10**
Kupffer cells												
Pigmentation	0/10	—	0/10	0/10	0/10	3/10	0/10	—	0/10	0/10	9/10**	10/10**

* $P \leq 0.05$; ** $P \leq 0.01$; —, not examined

Groups of five male and five female F344 rats were dosed intragastrically with furan (>99% pure) in corn oil for 12 days (days 1 through 5, 8 through 12 and at least 2 consecutive dosing days before terminal sacrifice) at doses of 0, 5, 10, 20, 40 or 80 mg/kg bw per day (males) and 0, 10, 20, 40, 80 or 160 mg/kg bw per day (females) starting when they were 47 days old. The rats were weighed at the start of the study and at days 8 and 16. Clinical observations were made twice daily. Complete necropsies were performed on all animals. All rats receiving 80 mg/kg bw or higher died within the first 8 days of the study. There were no other deaths during the study. Body weights of male rats receiving 20 or 40 mg/kg bw per day and female rats receiving 40 mg/kg bw per day were lower than those of control rats at the end of the study. The only clinical findings consistently observed in furan-dosed groups were inactivity or reduced activity. At necropsy, mottled and often enlarged livers were observed in the 20, 40 and 80 mg/kg bw per day male dose groups and the 40, 80 and 160 mg/kg bw per day female dose groups (NTP, 1993).

Groups of 10 male and 10 female F344 rats were dosed intragastrically with furan (>99% pure) in corn oil at doses of 0, 4, 8, 15, 30 and 60 mg/kg bw per day, 5 days/week, for 13 weeks, starting when they were 51 days old. The rats were observed twice daily, and clinical observations were recorded weekly. Body weights were taken at the start of the study and subsequently at weekly intervals. Surviving animals were killed at the end of the 13-week dosing period, and necropsies were performed on all animals. Weights of brain, heart, right kidney, liver, lungs and thymus were taken at necropsy. Complete histopathological examinations were made of all rats in the control groups and those receiving 30 or 60 mg/kg bw per day. Livers from all surviving animals were subjected to histopathological examination.

Nine male rats and four female rats from the highest dose group (60 mg/kg bw per day) died before the end of the study. Reduced activity and occasional irregular breathing were noticed in both male and female rats of the highest dose group. The final mean body weights of rats in the 15 and 30 mg/kg bw per day groups and the remaining female rats in the 60 mg/kg bw per day group were lower than those of the respective controls. Both absolute and relative liver weights were increased in males receiving 30 mg/kg bw per day and in females receiving 15, 30 or 60 mg/kg bw per day, in addition to being increased at the next lower doses (15 mg/kg bw per day and 8 mg/kg bw per day for males and females, respectively), without statistical significance for both parameters. Relative and absolute weights of the right kidney were also increased in female rats receiving 15, 30 or 60 mg/kg bw per day. Decreased thymus weights were also noted in males receiving 30 mg/kg bw per day (both absolute and relative weights) and in females receiving 60 mg/kg bw per day (absolute weights only). Other differences in organ weight measurements were considered secondary to the lower body weights in the dosed rats compared with controls.

Lesions associated with the administration of furan were most pronounced in the liver (Table 2), but the kidney, thymus, testes and ovaries were also affected. The most prominent change was bile duct hyperplasia, which occurred in all furan-dosed groups; the incidence and severity of the lesions showed a dose-related pattern. Cholangiofibrosis was also noted together with the bile duct hyperplasia.

Table 2. Incidences of selected non-neoplastic hepatic lesions in rats in the 13-week NTP (1993) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw	0 mg/kg bw	4 mg/kg bw	8 mg/kg bw	15 mg/kg bw	30 mg/kg bw	60 mg/kg bw
Biliary tract												
Cholangiofibrosis	0/10	4/10*	7/10**	10/10**	10/10**	10/10**	0/10	1/10	7/10**	10/10**	10/10**	9/10**
Hyperplasia	0/10	4/10*	9/10**	10/10**	10/10**	10/10**	0/10	7/10**	10/10**	10/10**	10/10**	9/10**
Hepatocytes												
Cytomegaly	0/10	0/10	0/10	8/10**	10/10**	10/10**	0/10	0/10	0/10	10/10**	10/10**	9/10**
Degeneration	0/10	0/10	7/10**	9/10**	10/10**	10/10**	0/10	0/10	1/10	10/10**	10/10**	10/10**
Necrosis	0/10	0/10	0/10	9/10**	10/10**	10/10**	0/10	0/10	0/10	8/10**	10/10**	10/10**
Hyperplasia, nodular	0/10	0/10	0/10	0/10	10/10**	10/10**	0/10	0/10	0/10	0/10	8/10**	9/10**
Kupffer cells												
Pigmentation	0/10	4/10*	6/10**	10/10**	10/10**	9/10**	0/10	2/10	8/10**	10/10**	10/10**	9/10**

* $P \leq 0.05$; ** $P \leq 0.01$

Lesions of hepatocytes included cytomegaly, degeneration, necrosis and nodular hyperplasia. In addition, greenish-brown pigmentation of Kupffer cells (presumably bile) was noted. The kidneys of nearly all the highest-dose (60 mg/kg bw per day) males and females and two of the 30 mg/kg bw per day females had dilated renal tubules up to approximately twice the normal diameter. Epithelial necrosis of the tubular epithelium was also present in most of the animals of the highest dose group. Atrophy of the testes, ovaries or thymus was observed in many of the male and female rats receiving 60 mg/kg bw per day. These changes were considered in the study report to be a result of general debilitation associated with furan toxicity (NTP, 1993).

A 90-day repeated-dose study was conducted on groups of 12 male and 12 female F344 rats, 5–6 weeks of age, dosed intragastrically with furan (99.9% purity) in corn oil at 0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day, 5 days/week, starting after a 12-day acclimatization period. Animals were observed for clinical signs twice daily on weekdays and once daily on weekends and holidays. The rats were weighed daily prior to dosing. Food consumption was measured on a weekly basis. At the end of the study, the animals were sacrificed and blood samples were collected from the abdominal aorta for measurement of haematology and clinical biochemistry parameters, including serum testosterone (reported in section 2.2.6) and thyroid hormone analysis. Necropsies were performed, and liver, kidneys, thymus, uterus, ovaries, ventral prostate, seminal vesicles and testes were weighed. Suspensions of thymus cells were prepared for lymphocyte phenotyping. In addition, furan residue analysis was conducted on samples of the median liver lobe. Samples of 26 organs and tissues were preserved for histopathological examination, including, for the liver, samples of the left lateral, median, right lateral and caudate lobes.

Dosing with furan over this dose range had no effect on body weights or food consumption. A dose-related increase in platelet count was noted in both male and female rats, which was statistically significant at 8.0 mg/kg bw per day in males and at 0.5 mg/kg bw per day and higher in females. Slight, dose-related, numerically lower red blood cell counts and haematocrits were noted in the male rats, without statistical significance, which were within the historical control range. Thymic lymphocyte populations shifted towards a decline in $CD4^+/CD8^+$ cells, with a corresponding increase in all the other populations of lymphocytes ($CD4^-/CD8^-$, $CD4^+/CD8^-$, $CD4^-/CD8^+$). Although these changes reflect altered thymocyte maturation, there were no accompanying changes in thymus weight or histology. Dose-related changes in clinical chemistry that achieved statistical significance were noted for numerous parameters related to liver function and included the following: decreases in ALT and AST activities (statistically significant for ALT in both sexes and for AST in females); slightly increased alkaline phosphatase (AP) activity in males only; decreased serum amylase (males, significant linear trend in females); increased serum albumin concentration (both sexes), decreased serum globulin concentration (males), increased albumin/globulin ratio (males) and increased total protein (females); slightly increased total cholesterol (males) and decreased serum triglycerides (both sexes); increased serum thyroxine (males) and triiodothyronine (significant linear trend in both sexes); and increased serum inorganic phosphorus, without notable effects on other electrolytes (both sexes). The concentration of unchanged furan in the liver increased 2-fold, 4-fold and 6-fold at doses of 0.5, 2.0 and 8.0 mg/kg bw per day, respectively.

Absolute liver weights were increased at the highest dose tested, 8.0 mg/kg bw per day, in both sexes. Macroscopic changes were observed in all of the livers of both male and female rats in this group. The caudate and left lateral lobes were primarily affected. The changes consisted of single or multiple small, firm, white and/or yellow nodules on the margins of these lobes; in some cases, the entire caudate lobe was affected, giving it an irregular surface. A greenish-yellowish discoloration of the surface and curling of the margin of the affected areas were also observed. Histological lesions of the liver were observed in both male and female rats (Table 3), starting at a dose of 0.12 mg/kg bw per day.

The severity and number of lesions increased with increasing dose of furan. The caudate lobe of the liver was most severely affected, followed by the left lateral lobe. At 0.12 mg/kg bw per day, rare to occasional apoptosis of hepatocytes, Kupffer cells filled with yellow pigment and microfoci of inflammatory cells were observed in the immediate subcapsular region, which were restricted to the dorsal margin and visceral surface (i.e. closest to the stomach) of the left lateral and caudate lobes. In the next higher dose group, 0.5 mg/kg bw per day, these lesions were noted at increased incidence, with the addition of a layer 1–2 cells thick interior to the subcapsular lesions of hepatocytes with cytomegaly, karyomegaly and dark, homogeneous, slightly basophilic cytoplasm. At 2.0 mg/kg bw per day, the layers of affected hepatocytes were increased up to five cells thick, with increased vacuolation of zone 3 (centrilobular) hepatocytes. In the more severely affected animals of this group, more extensive subcapsular and/or periportal proliferation of oval cells, bile duct hyperplasia, inflammatory cell infiltrate and mild fibrosis were observed. In the 8.0 mg/kg bw per day group, the lesions were similar to those at 2.0 mg/kg bw per day, but were more severe in nature and more extensive in distribution. Of note were the greater oval cell and bile duct epithelial cell hyperplasia, bile duct metaplasia and interstitial fibrosis, which formed the macroscopic raised lesions and replaced areas of normal hepatic parenchyma. The hyperplastic ductular structures varied in appearance from normal bile ducts, intestinal gland-like structures with flattened to tall columnar epithelium and interspersed mucus-producing cells, to cystic structures of variable size and shape, containing eosinophilic material and cell debris. These areas with their variable fibrotic and glandular components were classified by Elmore & Sirica (1993) (section 2.2.6) as primary hepatic intestinal-type adenocarcinomas (or intestinal-type cholangiocarcinomas) rather than hepatic cholangiocarcinomas. The lesions were focal (subcapsular and periportal), bridging portal tracts, in this way forming islands of normal hepatic parenchyma or completely replacing hepatic parenchyma. The caudate lobe was the most severely affected, but variable and considerable areas of the left lateral lobe were also affected, and the lesions were also evident in the margin of the right lateral lobe in a few animals (Gill et al., 2009).

In a separate report, immunohistochemical investigation of the livers from the above Gill et al. (2009) study revealed increased staining for proliferating cell nuclear antigen-positive nuclei in males at 0.12 mg/kg bw per day and in females at 0.5 mg/kg bw per day. Immunohistochemical-positive glutathione *S*-transferase-pi (GST-P) foci were found in males at 0.12 mg/kg bw per day and in females at 2.0 mg/kg bw per day (Curran et al., 2009).

Table 3. Incidences of selected non-neoplastic hepatic lesions in rats in the 13-week Gill et al. (2009) oral study

	Incidence of lesions											
	Males						Females					
	0 mg/kg bw	0.03 mg/kg bw	0.12 mg/kg bw	0.5 mg/kg bw	2.0 mg/kg bw	8.0 mg/kg bw	0 mg/kg bw	0.03 mg/kg bw	0.12 mg/kg bw	0.5 mg/kg bw	2.0 mg/kg bw	8.0 mg/kg bw
Biliary tract												
Cholangiofibrosis	0/12	0/12	0/12	0/12	2/12	12/12	0/12	0/12	0/12	0/12	0/12	11/12
Hyperplasia	0/12	0/12	0/12	0/12	6/12	12/12	0/12	0/12	0/12	0/12	1/12	12/12
Hepatocytes												
Apoptosis	3/12	4/12	6/12	12/12	12/12	12/12	2/12	0/12	1/12	10/12	12/12	12/12
Cytoplasmic basophilia	0/12	0/12	0/12	8/12	12/12	12/12	0/12	0/12	0/12	0/12	12/12	11/12
Anisokaryosis	0/12	0/12	0/12	0/12	10/12	12/12	0/12	0/12	0/12	0/12	12/12	10/12
Kupffer cells												
Pigmentation	1/12	1/12	5/12	10/12	12/12	12/12	2/12	0/12	3/12	12/12	12/12	12/12

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

(i) Oral route

Groups of 50 male and 50 female B6C3F1 mice that were on average 58 days of age at the start of the study were administered intragastric doses of furan in corn oil at 0, 8 or 15 mg/kg bw per day, 5 days/week, for 2 years. The mean body weights of both male and female high-dose mice were lower than those of the controls. From week 80 to the end of the study, survival of the male mice in both dose groups and the female mice of the high dose group were lower than in the control group due to moribund condition associated with liver neoplasms.

The strongest treatment-related effects were observed in the liver. The incidences of hepatocellular adenomas and carcinomas were significantly increased in mice dosed with furan (Table 4).

Table 4. Incidences of neoplastic liver lesions in mice in the 2-year NTP (1993) study

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Male			
2-year survival	33/50	17/50	16/50
Hepatocellular adenoma			
Overall rates[a]	20/50	33/50	42/50
Terminal rates[b]	15/33	12/17	16/16
Hepatocellular carcinoma			
Overall rates	7/50	32/50	34/50
Terminal rates	0/7	7/17	7/16
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[c]			
Overall rates	26/50	44/50	50/50
Terminal rates	15/33	16/17	16/16
Female			
2-year survival	29/50	25/50	2/50
Hepatocellular adenoma			
Overall rates	5/50	31/50	48/50
Terminal rates	5/29	17/25	2/2

Table 4 (contd)

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Hepatocellular carcinoma			
Overall rates	2/50	7/50	27/50
Terminal rates	1/29	3/25	1/2
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[d]			
Overall rates	7/50	34/50	50/50
Terminal rates	6/29	18/25	2/2

[a] Number of tumour-bearing animals/number of animals.
[b] Observed incidence at terminal necropsy.
[c] Historical incidence in 2-year NTP corn oil gavage studies for vehicle control groups (mean ± standard deviation): 210/599 (35.1% ± 11.0%), range 14–52%.
[d] Historical incidence: 60/597 (10.1% ± 4.3%), range 2–16%.

The incidences of numerous non-neoplastic hepatocellular lesions were also increased in dosed mice (Table 5). These lesions included hepatocyte cytomegaly, degeneration and necrosis; multifocal hyperplasia; cytoplasmic vacuolization; and biliary tract dilatation, fibrosis, hyperplasia and inflammation (NTP, 1993).

Table 5. Incidences of non-neoplastic liver lesions in mice in the 2-year NTP (1993) study

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Male			
Cytoplasmic vacuolization	8/50	24/50	36/50
Focal hyperplasia	1/50	44/50	49/50
Mixed cell cellular infiltration	2/50	23/50	29/50
Bile duct dilatation	0/50	0/50	6/50
Biliary tract			
Chronic inflammation	0/50	44/50	49/50
Fibrosis	0/50	45/50	49/50
Hyperplasia	0/51	46/50	49/50

Table 5 (contd)

	Incidence of lesions		
	0 mg/kg bw	8 mg/kg bw	15 mg/kg bw
Hepatocyte			
Cytomegaly	8/50	45/50	50/50
Degeneration	0/50	43/50	43/50
Necrosis	2/50	39/50	41/50
Kupffer cell pigmentation	2/50	43/50	50/50
Parenchymal focal atrophy	1/50	45/50	50/50
Female			
Cytoplasmic vacuolization	6/50	29/50	36/50
Focal hyperplasia	0/50	48/50	48/50
Lymphoid hyperplasia	27/50	33/50	42/50
Mixed cell cellular infiltration	8/50	23/50	32/50
Bile duct dilatation	0/50	1/50	11/50
Biliary tract			
Chronic inflammation	2/50	48/50	50/50
Fibrosis	0/50	47/50	50/50
Hyperplasia	0/50	47/50	50/50
Hepatocyte			
Cytomegaly	0/50	48/50	50/50
Degeneration	0/50	47/50	48/50
Necrosis	0/50	44/50	47/50
Kupffer cell pigmentation	5/50	48/50	50/50
Parenchymal focal atrophy	0/50	48/50	50/50

Male and female high-dose mice also had increased incidences of haematopoietic cell proliferation in the spleen, possibly secondary to hepatic inflammation (NTP, 1993).

Groups of female B6C3F1 mice (numbers in parentheses) 5–6 weeks of age were dosed intragastrically with furan (>99% pure) in corn oil (5 ml/kg bw) at 0 (50), 0.5 (100), 1.0 (100), 2.0 (50), 4.0 (50) and 8.0 (50) mg/kg bw per day, 5 days/week, for 2 years. At study start, group mean body weight was similar in all groups (10.1–19.6 g). Up to week 89, body weight was comparable in control animals and mice dosed with furan. At weeks 89 and 101, there was a significant decrease in mean group body weight in mice dosed with 8.0 mg/kg bw per day compared with

the mean body weight of control mice. At the terminal necropsy, mean group body weight was 42.5 g in control mice and 29.8, 39.7, 38.5, 42.4 and 38.6 g in mice dosed with 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, respectively. In mice dosed with 2.0 and 8.0 mg/kg bw per day, there were significant decreases in body weight gain from study initiation to the terminal necropsy; the decrease in body weight gain was not dose dependent (data not shown). There were no differences in the percentage of mice that survived to the scheduled terminal necropsy in any group dosed with furan relative to control mice. At the terminal necropsy, the per cent mortality in control mice was 34% compared with 31%, 40%, 19%, 36% and 46% in mice exposed to 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, respectively.

Complete macroscopic examinations were performed at necropsy. Livers were removed and weighed. Representative sections from the left, median, right posterior, right anterior and caudate lobes of the liver and sections of macrosopic lesions greater than 0.5 cm in size along with accompanying normal tissue were fixed in 10% neutral buffered formalin. At the terminal necropsy, mean group absolute and relative liver weights of mice dosed with 0.5, 1.0 and 2.0 mg/kg bw per day were comparable with those of control mice. In mice dosed with 4.0 and 8.0 mg/kg bw per day, absolute and relative liver weights were increased ($P = 0.007$ and 0.004, respectively) (data not shown).

The incidence of liver nodules at the terminal necropsy was significantly increased to 60% (15/25) and 100% (22/22) in mice exposed to 4.0 and 8.0 mg/kg bw per day ($P < 0.001$), respectively. The incidence of macroscopic liver nodules was 8% (2/25) in control mice and 17–20% in remaining groups of dosed mice. In animals that were necropsied before the terminal necropsy date, a significant increase in the incidence of visible liver nodules was found in mice exposed to 4.0 mg/kg bw per day (54%; 6/11) and 8.0 mg/kg bw per day (71%; 12/17) ($P = 0.032$ and 0.002, respectively). The incidence of gross liver nodules in control mice was 9% (1/11). Macroscopic nodules at the terminal necropsy were grouped by size. The largest nodules (≥0.5 mm) were present in mice exposed to 8.0 mg/kg bw per day (data not shown).

There was a dose-dependent trend in the incidence of foci of altered hepatocytes, adenomas, carcinomas, and adenomas or carcinomas ($P < 0.001$ for all) (Table 6). Increased incidences of hepatocellular foci developed in mice dosed with furan at 4.0 and 8.0 mg/kg bw per day ($P = 0.030$ and <0.001, respectively) compared with control mice, but there was no difference in the percentage of foci that were classified as basophilic, eosinophilic or clear-cell foci. Significant increases were found in the incidences of hepatocellular adenomas in groups dosed with 4.0 and 8.0 mg/kg bw per day ($P = 0.018$ and <0.001, respectively), carcinomas in mice dosed with 8.0 mg/kg bw per day ($P < 0.001$), and adenomas or carcinomas in those dosed with 4.0 and 8.0 mg/kg bw per day ($P = 0.015$ and <0.001, respectively) (Table 6). Only animals exposed to furan at 4.0 and 8.0 mg/kg bw per day had both adenomas and carcinomas (1/3 [3%] and 7/39 [18%], $P = 0.007$, respectively). There was a significant increase in the mean number of adenomas per animal in mice administered furan at dose levels of 4.0 and 8.0 mg/kg bw per day ($P = 0.015$ and <0.001, respectively) and adenomas or carcinomas at 8.0 mg/kg bw per day ($P = 0.002$) (data not shown). In general, latency period or time to first tumour was decreased with increasing dose of furan.

Table 6. Preneoplastic and neoplastic liver lesions in female mice exposed to furan in the 2-year Moser et al. (2009) study

	Incidence of lesions					
	Control	0.5 mg/kg bw	1.0 mg/kg bw	2.0 mg/kg bw	4.0 mg/kg bw	8.0 mg/kg bw
	n = 36	*n* = 72	*n* = 53	*n* = 41	*n* = 36	*n* = 39
Number of altered hepatocytes						
Overall rate[a]	3/36 (8%)	5/72 (7%)	4/53 (8%)	5/41 (12%)	10/36 (28%)	19/39 (49%)
Adjusted rate[b] (%)	10	7	9	14	32	55
Terminal rate[c]	3/25 (12%)	5/55 (9%)	4/36 (11%)	5/31 (16%)	10/25 (49%)	13/22 (59%)
Latency	725	725	725	725	725	590
Poly-3 test[d]		$P = 0.511$	$P = 0.620$	$P = 0.445$	$P = 0.030$[f]	$P < 0.001$[f]
Dose trend[e]	$P < 0.001$					
Hepatocellular adenoma						
Overall rate	3/36 (8%)	4/72 (6%)	4/53 (8%)	4/41 (10%)	11/36 (31%)	25/39 (64%)
Adjusted rate (%)	10	6	9	11	34	72
Terminal rate	3/25 (12%)	3/55 (5%)	4/36 (11%)	3/31 (10%)	8/25 (32%)	18/22 (82%)
Latency	725	713	725	525	511	489
Poly-3 test		$P = 0.407$	$P = 0.620$	$P = 0.596$	$P = 0.018$[f]	$P < 0.001$[f]
Dose trend	$P < 0.001$					

Table 6 (contd)

	Incidence of lesions					
	Control	0.5 mg/kg bw	1.0 mg/kg bw	2.0 mg/kg bw	4.0 mg/kg bw	8.0 mg/kg bw
	n = 36	*n* = 72	*n* = 53	*n* = 41	*n* = 36	*n* = 39
Hepatocellular carcinoma						
Overall rate	0/36 (0%)	4/72 (6%)	2/53 (4%)	1/41 (2%)	2/36 (6%)	11/39 (28%)
Adjusted rate (%)	0	6	4	3	6	33
Terminal rate	0/25 (0%)	2/55 (4%)	1/36 (3%)	1/31 (3%)	1/25 (4%)	6/22 (27%)
Latency	NA[g]	439	566	726	511	652
Poly-3 test		*P* = 0.208	*P* = 0.322	*P* = 0.532	*P* = 0.244	*P* < 0.001[f]
Dose trend	*P* < 0.001					
Hepatocellular adenoma or carcinoma						
Overall rate	3/36 (8%)	8/72 (11%)	6/53 (11%)	5/41 (12%)	12/36 (33%)	29/39 (74%)
Adjusted rate (%)	10	12	13	14	35	63
Terminal rate	3/25 (12%)	5/55 (9%)	5/36 (14%)	4/31 (13%)	9/25 (36%)	14/22 (64%)
Latency	725	439	566	525	511	489
Poly-3 test		*P* = 0.516	*P* = 0.449	*P* = 0.455	*P* = 0.015[f]	*P* < 0.001[f]
Dose trend	*P* < 0.001					

Table 6 (contd)

n, number examined microscopically

[a] Number of tumour-bearing animals/total number of animals evaluated microscopically.

[b] Number of tumour-bearing animals/effective number of animals, i.e. number of animals alive at first occurrence of this tumour type in any of the groups.

[c] Observed incidence at terminal kill.

[d] Beneath the control incidence are the *P*-values associated with the trend test. Beneath the dosed group incidence are the *P*-values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumours in animals dying prior to terminal kill as non-fatal.

[e] Dose-related trend as calculated by a modified Cochran-Armitage linear trend test.

[f] Significant increase compared with control mice ($P < 0.05$).

[g] Not applicable; no tumours in animal group.

Among mice examined at the terminal necropsy at 24 months, the incidence of foci, adenomas, carcinomas, and adenomas or carcinomas was similar in controls and animals exposed to furan at 0.5, 1.0 and 2.0 mg/kg bw per day. Increases at 4.0 mg/kg bw per day in the prevalence of foci ($P = 0.030$), adenomas ($P = 0.018$), and adenomas or carcinomas ($P = 0.015$) and at 8.0 mg/kg bw per day in the prevalence of foci, adenomas, carcinomas, and adenomas or carcinomas ($P < 0.001$ for all) were observed (Table 6) (Moser et al., 2009).

(ii) Intraperitoneal route

Groups of B6C3F1 infant mice were dosed intraperitoneally with furan in tricaprylin by two different regimens: either 400 mg/kg bw (the lethal dose for 10% of mice, or LD_{10}) was administered once on postnatal day 15 to a group of 215 mice, or 200 mg/kg bw (the lethal dose for 5% of mice, or LD_5) was administered 6 times, once each on postnatal days 3, 6, 9, 12, 15 and 18, to a group of 78 mice. Corresponding control groups, 52 and 79 mice, respectively, received tricaprylin only. Mice were randomly selected and killed at 16 time points between 28 and 95 weeks after dosing for collection of benign and malignant liver tumours. The total number of mice killed was 215 in the 400 mg/kg bw furan group, 52 in the single-dose vehicle (tricaprylin) group, 78 in the 6 × 200 mg/kg bw furan group and 79 in the 6 × vehicle (tricaprylin) group.

Body weights were consistently slightly greater, starting at week 14, in mice administered furan at 200 mg/kg bw 6 times than in mice in the other three groups (data not shown). At necropsy, livers were excised, weighed and examined for visible lesions. For any tumour larger than 0.5 cm in diameter, a representative section was submitted for histological analysis. There was no statistically significant increased incidence in hepatocellular tumours in mice given a single preweaning dose of 400 mg/kg bw over the single-dose vehicle controls during the study (Table 7). The overall frequency of hepatocytic neoplasia was 27% in the 400 mg/kg bw furan group compared with 15% in the single-dose controls, and the overall liver tumour multiplicity was increased. In mice given six neonatal furan doses of 200 mg/kg bw, there was an increased incidence of hepatocellular neoplasms compared with the multiple-dose vehicle control group as well as the 400 mg/kg bw furan group. The tumour multiplicity was 1.1 in the 6 × 200 mg/kg bw furan group and 0.41 in the six-dose vehicle control group (Johansson et al., 1997).

(b) Rats

(i) Oral route

Groups of 70 male and 70 female F344 rats that were on average 51 days of age at the start of the study were administered intragastric doses of furan in corn oil at 0, 2, 4 or 8 mg/kg bw per day, 5 days/week, for 2 years. After dosing for 9 and 15 months, 10 rats from each group were necropsied. Mean body weights of male rats that received 8 mg/kg bw per day were lower than those of controls from approximately week 73 to the end of the study. Survival of male and female rats that received 8 mg/kg bw per day was lower than that of controls from approximately week 85 to the end of the study as a result of moribund condition associated with liver and biliary tract neoplasms and mononuclear cell leukaemia.

Table 7. Liver tumour incidence and multiplicity in the Johansson et al. (1997) study

Treatment	Overall incidence			Mean (± SE) liver tumour (adenomas and carcinomas) multiplicity	
	Adenomas	Carcinomas	Adenomas and carcinomas	All mice	Tumour-bearing mice
Vehicle control	5/52	3/52	8/52	0.17 (± 0.06)	1.12 (± 0.12)
(1 × tricaprylin)	(9.6%)	(5.8%)	(15.4%)		
Furan—1 dose	47/215	16/215	58/215	0.40 (± 0.05)	1.50 (± 0.10)
(1 × 400 mg/kg bw)	(21.9%)	(7.4%)	(27.0%)		
Vehicle control	14/79	8/79	21/79	0.41 (± 0.10)	1.52 (± 0.22)
(6 × tricaprylin)	(17.7%)	(10.1%)	(26.6%)		
Furan—6 doses	31/78*	14/78**	40/78*	1.09 (± 0.18)***	2.12 (±0.25)†
(6 × 200 mg/kg bw)	(39.7%)	(17.9%)	(51.3%)		

SE, standard error

* $P = 0.001$ compared with 6 × tricaprylin control and 1 × 400 mg/kg bw furan group. The statistical analysis was based on logistic regression.

** $P = 0.001$ compared with 1 × 400 mg/kg bw furan group. The statistical analysis was based on logistic regression.

*** $P < 0.001$ compared with 6 × tricaprylin control and 1 × 400 mg/kg bw furan group. The statistical analysis was based on Poisson regression of tumour counts on days on study.

† $P = 0.013$ compared with 6 × tricaprylin control and $P < 0.001$ compared with 1 × 400 mg/kg bw furan group. The statistical analysis was based on Poisson regression of tumour counts on days on study.

The most marked dosing-related effects were observed in the liver (Table 8). In all dosed groups, cholangiocarcinoma of the liver was observed in many rats of each sex at the 9- and 15-month interim evaluations. At 2 years, incidences of cholangiocarcinoma were 86%, 96% and 98% in furan groups. Incidences of hepatocellular adenomas or carcinomas (combined) were increased in both male and female rats after 2 years of furan exposure, and incidences of hepatocellular adenomas were significantly increased in female rats. The incidences of mononuclear cell leukaemia were increased in male and female rats that received 4 or 8 mg/kg bw per day, and the incidence in the 8 mg/kg bw per day groups of each sex exceeded the historical control ranges for corn oil gavage studies (NTP, 1993).

Non-neoplastic liver lesions, including biliary tract fibrosis, hyperplasia and chronic inflammation and hepatocyte cytomegaly, cytoplasmic vacuolization, degeneration, nodular hyperplasia and necrosis were abundant in all rats administered furan (Table 9). Also, at 9 months, male rats at all doses showed an

Table 8. Incidences of neoplastic lesions in F344/N rats in the 2-year NTP (1993) study

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Male				
2-year survival	33/50	28/50	26/50	16/50
Cholangiocarcinoma				
Overall rates[a]	0/50	43/50	48/50	49/50
Terminal rates[b]	0/33	28/28	26/26	16/16
Hepatocellular adenoma				
Overall rates	1/50	4/50	18/50	27/50
Terminal rates	0/33	4/28	13/26	8/16
Hepatocellular carcinoma				
Overall rates	0/50	1/50	6/50	18/50
Terminal rates	0/33	1/28	5/26	5/16
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[c]				
Overall rates	1/50	5/50	22/50	35/50
Terminal rates	0/33	5/28	16/26	10/16
Mononuclear cell leukaemia[d]				
Overall rates	8/50	11/50	17/50	25/50
Terminal rates	4/33	6/28	8/26	8/16
Female				
2-year survival	34/50	32/50	28/50	19/50
Cholangiocarcinoma				
Overall rates	0/50	49/50	50/50	48/50
Terminal rates	0/34	32/32	28/28	19/19
Hepatocellular adenoma				
Overall rates	0/50	2/50	4/50	7/50
Terminal rates	0/34	1/32	3/28	6/19
Hepatocellular carcinoma				
Overall rates	0/50	0/50	0/50	1/50
Terminal rates[e]	—	—	—	—

Table 8 (contd)

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Hepatocellular adenoma, hepatocellular carcinoma or hepatocellular adenoma and hepatocellular carcinoma[f]				
Overall rates	0/50	2/50	4/50	8/50
Terminal rates	0/34	1/32	3/28	6/19
Mononuclear cell leukaemia[g]				
Overall rates	8/50	9/50	17/50	21/50
Terminal rates	1/34	5/32	9/28	7/19

[a] Number of tumour-bearing animals/number of animals necropsied.
[b] Observed incidence at terminal kill.
[c] Historical incidence for 2-year NTP corn oil gavage studies for vehicle control groups (mean ± standard deviation): 19/770 (2.5% ± 2.8%), range 0–10%.
[d] Historical incidence: 164/770 (21.3% ± 8.9%), range 4–38%.
[e] Data not provided.
[f] Historical incidence: 9/770 (1.2% ± 2.7%), range 0–10%.
[g] Historical incidence: 206/770 (26.8% ± 7.0%), range 16–38%.

increased incidence of bone marrow hyperplasia and congestion/haematopoietic cell proliferation of the spleen, along with lower red blood cell count, haemoglobin level and haematocrit, at the high dose. Females showed bone marrow hyperplasia at all doses and splenic haematopoietic cell proliferation in the spleen at the intermediate and high doses. Haematology was not altered.

A 2-year stop-exposure study was conducted in male F344/N rats 47–61 days of age at the start of the study. Groups of 50 rats were dosed intragastrically either with corn oil (vehicle control) or with furan in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks and then maintained for up to 90 additional weeks without further furan administration. Groups of 10 animals were necropsied at the end of the 13-week dosing phase, after 9 months and after 15 months. Further, 6 animals that died in a moribund condition between the 9- and 15-month terminations and 14 animals that died between the 15-month termination and the end of the 2-year study were evaluated. Histopathological evaluation was performed on the livers from all 13-week interim rats; on livers and kidneys from all 9-month interim rats; and on livers, kidneys and lungs from all 15-month interim rats.

The incidences of neoplastic and non-neoplastic liver lesions are presented in Table 10. No liver lesions were found in the vehicle control animals that were examined at the 9- and 15-month interim evaluations; a single control male had cytoplasmic vacuolization of hepatocytes. In dosed rats, no liver neoplasms were

Table 9. Incidences of non-neoplastic liver lesions in F344/N rats in the 2-year NTP (1993) study

	Incidence of lesions			
	Vehicle control	2 mg/kg bw	4 mg/kg bw	8 mg/kg bw
Male				
Multilocular cyst	0/50	1/50	17/50	24/50
Biliary tract				
Chronic focal inflammation	0/50	44/50	48/50	49/50
Cyst	0/50	44/50	47/50	49/50
Focal fibrosis	0/50	44/50	48/50	49/50
Focal hyperplasia	0/50	44/50	48/50	49/50
Metaplasia	0/50	44/50	48/50	49/50
Hepatocyte				
Cytomegaly	0/50	35/50	46/50	49/50
Cytoplasmic vacuolization	1/50	39/50	45/50	49/50
Focal degeneration	0/50	33/50	46/50	49/50
Focal hyperplasia	0/50	30/50	46/50	49/50
Focal necrosis	0/50	32/50	46/50	49/50
Kupffer cell focal pigmentation	0/50	44/50	48/50	49/50
Female				
Multilocular cyst	0/50	6/50	2/50	12/50
Biliary tract				
Chronic focal inflammation	0/50	49/50	50/50	49/50
Cyst	0/50	49/50	50/50	46/50
Focal fibrosis	0/50	49/50	50/50	49/50
Focal hyperplasia	0/50	49/50	50/50	49/50
Metaplasia	0/50	49/50	50/50	49/50
Hepatocyte				
Cytomegaly	0/50	44/50	50/50	49/50
Cytoplasmic vacuolization	0/50	43/50	49/50	47/50
Focal degeneration	0/50	35/50	49/50	47/50
Focal hyperplasia	0/50	32/50	47/50	46/50
Focal necrosis	0/50	18/50	46/50	47/50
Kupffer cell focal pigmentation	0/50	49/50	50/50	48/50

Table 10. Neoplastic and non-neoplastic liver lesions in male rats in the NTP (1993) stop-exposure study

	Incidence of lesions				
	13 weeks	9 months	9–15 months	15 months	After 15 months
Number of animals examined	10	10	6[a]	10	14[a]
Neoplasms					
Cholangiocarcinoma	0/10	10/10	6/6	10/10	14/14
Hepatocellular carcinoma	0/10	0/10	0/6	2/10	4/14
Non-neoplastic lesions					
Biliary tract					
Fibrosis, multifocal	10/10	10/10	6/6	10/10	14/14
Hyperplasia, multifocal	10/10	10/10	6/6	10/10	14/14
Inflammation, chronic, multifocal	0/10	10/10	6/6	10/10	14/14
Cysts, multiple	0/10	10/10	4/6	10/10	14/14
Hepatocytes					
Cytomegaly	10/10	10/10	6/6	10/10	14/14
Degeneration, multifocal	10/10	10/10	6/6	10/10	14/14
Hyperplasia, nodular, multifocal	10/10	10/10	6/6	10/10	14/14
Necrosis, multifocal	10/10	10/10	6/6	10/10	14/14
Vacuolization, cytoplasmic	10/10	10/10	6/6	10/10	14/14
Kupffer cells					
Pigmentation, multifocal	10/10	10/10	6/6	10/10	14/14

[a] Animals died in a moribund condition between the interim evaluations.

present at the end of dosing, but by 9 months, all of the rats (40/40) that survived had cholangiocarcinomas; and in 15% (6/40) of the rats, hepatocellular carcinomas developed, of which the first two were observed at the 15-month evaluation (NTP, 1993).

Two separate experiments were performed in young adult male F344 rats (160–190 g). In the first experiment, 12 rats were dosed intragastrically with furan (>99% pure) in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks. In the second experiment, three groups of 10 rats each were dosed with furan at 30 mg/kg bw per day as above, for 6, 9 or 12 weeks. In both experiments, the rats

were sacrificed at 16 months after initiation of the furan dosing. A complete necropsy was performed on each rat. Samples of all resulting hepatic and non-hepatic tumours, as well as random non-tumorous portions of each liver lobe, were processed for histopathology. The furan-dosed rats in both experiments exhibited very high incidences of hepatic adenocarcinoma at 16 months. In the first experiment, this incidence was 90%, and in the second experiment, it was 75% and 71% for rats that received furan for 9 and 12 weeks, respectively. Even when furan was administered for only 6 weeks, 44% of the dosed rats were found to have a hepatic adenocarcinoma at the end of the 16-month experimental period (Elmore & Sirica, 1993) (see section 2.2.3 for more details).

2.2.4 Genotoxicity

The results of genotoxicity studies with furan are summarized in Table 11, and those with its reactive metabolite BDA (see section 2.1.2) in Table 12. In the in vitro testing, an S9 fraction was used for bioactivation in many studies. The S9 was usually obtained from Aroclor 1254–induced rodent liver. Aroclor 1254 has been reported to be a poor inducer of CYP2E1 (Borlak & Thum, 2001; Escobar-Garcia et al., 2001; Mori et al., 2001), the CYP that is mainly responsible for the metabolism of furan to BDA. Thus, negative results could be due to deficient metabolism.

Table 11. Results of furan genotoxicity testing

End-point	Test system	Concentration/dose	Result	Reference
In vitro DNA alteration				
Strand breaks	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
DNA repair	Primary rat hepatocytes	Up to 681 µg/ml, −S9 activation	Negative	Wilson et al. (1992)
DNA repair	Primary mouse hepatocytes	Up to 681 µg/ml, −S9 activation	Negative	Wilson et al. (1992)
Bacterial gene mutation				
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate, +S9 activation	Negative	Mortelmans et al. (1986); NTP (1993)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate, −S9 activation	Negative	Mortelmans et al. (1986); NTP (1993)

Table 11 (contd)

End-point	Test system	Concentration/ dose	Result	Reference
In vitro mammalian gene mutation assays				
Thymidine kinase gene mutation	Mouse L5178Y lymphoma cells	125–3800 µg/ml, −S9 activation	Positive	McGregor et al. (1988); NTP (1993)
Thymidine kinase gene mutation	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
In vitro chromosomal alterations in mammalian cells				
SCEs	CHO cells	Up to 13 614 µg/ml, −S9 activation	Negative	Stich et al. (1981)
SCEs	CHO cells	Up to 13 614 µg/ml, +S9 activation	Positive	Stich et al. (1981)
SCEs	CHO cells	1.6–160 µg/ml, −S9 activation	Positive	NTP (1993)
SCEs	CHO cells	16–500 µg/ml, +S9 activation	Positive	NTP (1993)
Chromosomal aberrations	CHO cells	Up to 13 614 µg/ml, −S9 activation	Negative	Stich et al. (1981)
Chromosomal aberrations	CHO cells	Up to 13 614 µg/ml, +S9 activation	Positive	Stich et al. (1981)
Chromosomal aberrations	CHO cells	100–500 µg/ml, −S9 activation	Positive	NTP (1993)
Chromosomal aberrations	CHO cells	160–1000 µg/ml, +S9 activation	Positive	NTP (1993)
Micronucleus induction	Human lymphocyte cultures	136–1361 µg/ml, −S9 activation	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
Micronucleus induction	Human lymphocyte cultures	136–1361 µg/ml, +S9 activation	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)

Table 11 (contd)

End-point	Test system	Concentration/ dose	Result	Reference
Micronucleus induction	Mouse L5178Y $tk^{+/-}$ lymphoma cells	0.225–3.1 mmol/l, −S9 activation	Negative	Kellert et al. (2008b)
In vivo DNA alteration				
DNA repair	Mouse hepatocytes	10–200 mg/kg bw intragastrically for 2 h; 10–100 mg/kg intragastrically for 12 h	Negative	Wilson et al. (1992)
DNA repair	Rat hepatocytes	5–100 mg/kg bw intragastrically for 2 h and 12 h	Negative	Wilson et al. (1992)
Strand breaks	B6C3F1 mouse splenocytes	2–15 mg/kg bw intragastrically for 4 weeks, 24 h after last dosing; 15–250 mg/kg bw intragastrically, single dose for 3 h	Negative	Leopardi et al. (2010)
In vivo chromosomal alterations				
SCEs	B6C3F1 mouse bone marrow	87.5–350 mg/kg bw for 17 h; 62.5–250 mg/kg bw for 36 h	Positive in 250 mg/kg bw for 36 h	NTP (1993)
SCEs	F344 rat bone marrow	0.1–2.0 mg/kg bw for 5 days or 4 weeks	Negative	Mosesso (2009)
Chromosomal aberrations	B6C3F1 mouse bone marrow	87.5–350 mg/kg bw for 23 h; 25–100 mg/kg bw for 48 h	Negative	NTP (1993)
Chromosomal aberrations	F344 rat bone marrow	0.1–2.0 mg/kg bw for 5 days or 4 weeks	Negative	Mosesso (2009)
Micronuclei	B6C3F1 mouse splenocytes	2–15 mg/kg bw for 4 weeks, 24 h after last dosing	Positive	Leopardi et al. (2010)
Micronuclei	B6C3F1 mouse splenocytes	15–250 mg/kg bw single dose for 3 h	Statistically insignificant trend	Leopardi et al. (2010)

Table 11 (contd)

End-point	Test system	Concentration/dose	Result	Reference
Micronuclei	Balb/C mouse erythrocytes	50–300 mg/kg bw intraperitoneally; 150 and 225 mg/kg bw subcutaneously	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
Micronuclei	CBA mouse erythrocytes	225 mg/kg bw intraperitoneally	Negative	Durling, Svensson & Abramsson-Zetterberg (2007)
In vivo mutagenicity				
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	10 000 ppm feed	Negative	NTP (1993); Foureman et al. (1994)
		25 000 ppm injection	Negative	

CHO, Chinese hamster ovary; ppm, parts per million; SCE, sister chromatid exchange

Table 12. Results of BDA genotoxicity testing

End-point	Test system	Concentration	Result	Reference
In vitro DNA alteration				
Strand breaks	CHO-K1 cells	0.17–1.5 mmol/l	Positive	Marinari, Ferro & Sciaba (1984)
Strand breaks	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Positive	Kellert et al. (2008b)
Bacterial gene mutation				
Reverse mutation	*Salmonella typhimurium* TA97, TA98, TA100, TA102, TA104	1.4–4.3 µmol/plate (95.3–292.7 µg/plate)	Positive in TA104, negative in the other strains	Peterson, Naruko & Predecki (2000)

Table 12 (contd)

End-point	Test system	Concentration	Result	Reference
Gene mutation in mammalian cells				
Thymidine kinase gene mutation	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Positive	Kellert et al. (2008b)
Chromosomal alterations in mammalian cells				
Micronucleus	Mouse L5178Y $tk^{+/-}$ lymphoma cells	6.3–50 µmol/l	Negative	Kellert et al. (2008b)

(a) In vitro assays

(i) Furan

Furan was tested without addition of S9 in vitro in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of strand breaks using the alkaline comet assay at the nominal concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l. The "effective" concentrations of furan (i.e. corrected for losses through evaporation and diffusion into the dish material) were estimated to have been 0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l, respectively. Furan did not affect DNA migration under the test conditions. Furan was non-toxic to the cells (Kellert et al., 2008b).

Unscheduled DNA synthesis (UDS) as an indicator for DNA repair was measured in vitro in primary rat and mouse hepatocytes after exposure to furan concentrations up to 10 mmol/l (681 µg/ml). No increased UDS was found in these experiments (data not shown); however, the authors ascribe only limited importance to these findings because of the rapid loss of furan (due to its high vapour pressure) from the medium under these conditions (Wilson et al., 1992).

In mutagenicity assays using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, furan was found not to induce reverse mutations with and without preincubation with Aroclor 1254–induced rat or hamster liver S9 at concentrations of 33, 100, 333, 1000, 3333 and 10 000 µg/plate (Mortelmans et al., 1986; NTP, 1993).

In mouse L5178Y lymphoma cells, furan induced trifluorothymidine resistance at concentrations of 1139 µg/ml (2600 µg/ml in a batch of less respondent cells) and above without S9 activation; it was not tested with S9 (McGregor et al., 1988; NTP, 1993).

Furan was tested in the L5178Y $tk^{+/-}$ mouse lymphoma gene mutation assay at concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l (0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l effective concentrations, respectively) without addition of S9. Furan gave negative results for the induction of mutations under the test conditions (Kellert et al., 2008b).

The induction of sister chromatid exchanges (SCEs) of furan at concentrations of up to 200 mmol/l (13 614 µg/ml) was measured in Chinese hamster ovary (CHO) cells with and without the addition of S9 mixture derived from Aroclor 1254–induced rat liver. Furan induced SCEs only in the presence of S9. The data are presented in a graph only and do not allow the quantification of the response; no lowest effective dose is indicated. However, a dose-dependent response was observed (Stich et al., 1981). Interestingly, some of the concentrations applied are far above the concentrations reported in other studies as being too cytotoxic to evaluate.

In CHO cells, furan was tested for the induction of SCEs at concentrations of 1.6, 5, 16, 50 and 160 µg/ml without activation with Aroclor 1254–induced rat liver S9 and at concentrations of 16, 50, 160 and 500 µg/ml with S9 activation. With S9 activation, SCEs were induced only at the highest concentration of 500 µg/ml (NTP, 1993). Without S9 activation, in one trial, a significant induction of SCEs was measured over the whole range of concentrations, whereas in another trial in the same study, in a batch of less respondent cells also without S9 activation, furan induced a significant amount of SCEs only at the concentration of 160 µg/ml and not at the lower concentrations tested (see above).

The induction of chromosomal aberrations at furan concentrations of up to 200 mmol/l (13 614 µg/ml) was measured in CHO cells with and without the addition of S9 mixture derived from Aroclor 1254–induced rat liver. Furan induced chromosomal aberrations only in the presence of S9. The data are presented in a graph only and do not allow the quantification of the response; no lowest effective dose is indicated. However, a dose-dependent response was observed (Stich et al., 1981).

Furan induced chromosomal aberrations at concentrations of 100, 160, 300 and 500 µg/ml without S9 activation. With S9 activation, furan was tested at the concentrations of 160, 300, 500 and 1000 µg/ml; furan significantly increased chromosomal aberrations at the high concentrations of 500 and 1000 µg/ml (NTP, 1993). These results differ from the results of the aforementioned study by Stich et al. (1981) in which furan was found to have clastogenic effects in CHO cells only in the presence of S9.

In the NTP (1993) report, it is mentioned that the evaluation of the high doses tested in the CHO assays was limited by toxicity. It has also been suggested that the SCEs and chromatid aberrations could be due to lysosome breakdown secondary to cytotoxicity and not from direct chemical action on DNA (NTP, 1993).

The formation of micronuclei was tested in human lymphocyte cultures with furan at concentrations up to 100 mmol/l (6.807 mg/ml). Micronuclei were not formed to a statistically significant extent in the presence or absence of S9 mixture. Concentrations of 25 mmol/l (1702 µg/ml) and above could not be evaluated due to furan's cytotoxicity (Durling, Svensson & Abramsson-Zetterberg, 2007).

Furan was tested without addition of S9 in vitro in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of micronuclei at concentrations of 0, 0.25, 0.5, 1, 2 and 4 mmol/l (0, 0.225, 0.45, 0.9, 1.6 and 3.1 mmol/l effective concentrations, respectively). It gave negative results under the test conditions over the whole dose range tested (Kellert et al., 2008b).

(ii) BDA

BDA, also referred to as malealdehyde, was tested for induction of single-strand breaks in CHO-K1 cells. Concentrations of 0.17, 0.5 and 1.5 mmol/l were applied for 90 min, and breaks were assessed by alkaline elution. BDA increased elution at 0.5 and 1.5 mmol/l in a dose-related manner (Marinari, Ferro & Sciaba, 1984).

BDA was tested in the comet assay in L5178Y $tk^{+/-}$ mouse lymphoma cells for the induction of DNA damage at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. BDA slightly increased (1.6-fold) the DNA migration at 25 µmol/l. At 50 µmol/l, BDA was toxic (viability was reduced to below 50%) (Kellert et al., 2008b).

BDA was tested in several *Salmonella typhimurium* strains for the induction of reverse mutation. BDA tested positive in strain TA104 (which is sensitive to aldehydes) and negative in strains TA97, TA98, TA100 and TA102. The induction of reverse mutations was dose dependent (1000 ± 180 revertants/µmol per plate). In strain TA104, the concentrations 1.4, 1.7, 2.1, 2.9 and 4.3 µmol/plate were tested, of which 2.9 and 4.3 µmol/plate were toxic. Incubation of BDA with GSH prior to the addition of the cells prevented the toxic and genotoxic activity of BDA. BDA was not mutagenic in the other strains at non-toxic concentrations (Peterson, Naruko & Predecki, 2000).

BDA was tested in the mouse lymphoma thymidine kinase gene mutation assay at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. BDA produced a dose-dependent induction of thymidine kinase gene mutations (Kellert et al., 2008b).

BDA was tested for the induction of micronuclei in mouse L5178Y lymphoma cells at concentrations of 0, 6.3, 12.5, 25 and 50 µmol/l. It did not induce micronucleus formation under the conditions tested (Kellert et al., 2008b).

(b) In vivo assays

DNA repair activity in liver after furan dosing of male B6C3F1/CrlBR mice and male F344/CrlBR rats was determined by measurement of UDS in isolated hepatocytes. Mice were dosed intragastrically with furan at 10, 50 or 200 mg/kg bw 2 h before hepatocyte isolation or with 10, 50 or 100 mg/kg bw 12 h before hepatocyte isolation. Rats were dosed intragastrically with 5, 30 or 100 mg/kg bw 2 and 12 h before hepatocyte isolation. No increase in UDS in cultured hepatocytes was observed under any of the tested conditions (Wilson et al., 1992).

To investigate the genotoxicity of furan in vivo, splenocytes of male B6C3F1 mice after acute and repeated dosing were evaluated for DNA damage using the alkaline comet assay and immunofluorescence detection of foci of phosphorylated histone H2AX. The spleen was selected as target tissue due to the capability of quiescent splenocytes to accumulate DNA damage induced by repeated-dose exposure. Animals in the repeated-dose exposure groups were dosed with furan in corn oil at 2, 4, 8 or 15 mg/kg bw per day by intragastric administration for 4 weeks; animals in the acute studies received furan in corn oil at 15, 100 or 250 mg/kg bw per day by intragastric administration. The rats were sacrificed 24 h after the last dosing in the repeated-dose groups and 3 h after dosing in the acute groups. To

increase the sensitivity of the assay, splenocytes of furan-dosed mice in the repeated-dose exposure study were additionally incubated with a DNA repair inhibitor. Furan at 8 and 15 mg/kg bw increased γ-H2Ax foci, but did not produce DNA damage (Leopardi et al., 2010).

To assess the frequency of SCEs in bone marrow after furan dosing, male B6C3F1 mice (five animals per dose group) were injected intraperitoneally with 87.5, 175 and 350 mg/kg bw furan in corn oil (0.4 ml) 23 h before harvest and 25, 50 and 100 mg/kg bw 48 h before sacrifice and harvest. Solvent control mice received equivalent injections of corn oil only; the mice in the positive control study received injections of dimethylbenzanthracene at 100 mg/kg bw. No increase of SCEs was observed in the bone marrow of mice dosed with furan (NTP, 1993).

The induction of SCEs was studied in rat bone marrow of male F344/N rats dosed orally with furan for 5 and 28 days and 28 days followed by 2-week recovery at doses of 0.1, 0.5 and 2.0 mg/kg bw. A slight reduction in mitotic index was observed at the highest dose level after 5 days and 28 days plus recovery. No effect on SCEs was found (Mosesso, 2009).

The frequency of chromosomal aberrations in bone marrow of male B6C3F1 mice (10 per group) after furan dosing was assessed in the NTP (1993) bioassay. The mice were dosed intraperitoneally with furan at 87.5, 175 and 350 mg/kg bw 17 h before sacrifice and harvest and at 62.5, 125 and 250 mg/kg bw 36 h before sacrifice and harvest. The 36 h dosing experiments were repeated. Only at the highest dose tested (i.e. 250 mg/kg bw) in the 36 h experiments was an increase in aberrations observed (similar to the positive control response) (NTP, 1993).

The induction of chromosomal aberrations was studied in rat bone marrow of male F344/N rats dosed orally with furan for 5 and 28 days and 28 days followed by a 2-week recovery at doses of 0.1, 0.5 and 2.0 mg/kg bw. A slight reduction in mitotic index was observed at the highest dose level after 5 days and 28 days plus recovery. No effect on chromosomal aberrations was found (Mosesso, 2009).

The induction of micronuclei was also evaluated after both repeated and single dosing. Animals in the repeated-dose exposure groups were dosed with furan in corn oil at 2, 4, 8 or 15 mg/kg bw per day by intragastric administration for 4 weeks; animals in the acute studies received furan in corn oil at 15, 100 or 250 mg/kg bw per day by intragastric administration. The rats were sacrificed 24 h after the last dosing in the repeated-dose groups and 3 h after dosing in the acute dose groups. In the repeated-dose exposure studies, furan significantly induced the formation of micronuclei at doses of 4 mg/kg bw per day ($P < 0.005$) and above ($P < 0.001$). In the acute study, a statistically insignificant dose-related trend of increased micronuclei induction was observed (Leopardi et al., 2010).

The induction of micronuclei in erythrocytes of male Balb/C and CBA mice after intraperitoneal and subcutaneous administration was investigated. Furan was administered by intraperitoneal injection to Balb/C mice at 0, 50, 75, 90, 110, 125, 150, 175, 200, 250 and 300 mg/kg bw and to CBA mice at 225 mg/kg bw. Further Balb/C mice were dosed with furan at 0, 150 and 275 mg/kg by subcutaneous injection. No increased level of micronucleated erythrocytes was detected in any of the in vivo experiments (Durling, Svensson & Abramsson-Zetterberg, 2007).

Furan was tested for mutagenicity in the *Drosophila melanogaster* sex-linked recessive lethal assay. When fed at 10 000 parts per million (ppm) or injected at 25 000 ppm, it produced 21% and 28% mortality, respectively, but no mutagenicity (NTP, 1993; Foureman et al., 1994).

2.2.5 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies were found. Hormonal effects are reported in section 2.2.6.

2.2.6 Special studies

(a) Covalent binding

(i) Furan

Intragastric administration to male F344 rats of a single 8 mg/kg bw dose of diluted [2,5-^{14}C]furan, representing 370 kBq/kg bw, resulted in binding to protein in liver and to lesser degrees in kidney and blood. The level of binding increased with repeated 8 mg/kg bw doses over 8 days. In this study, DNA binding was not found in liver at 24 h after intragastric dosing of [2,5-^{14}C]furan at 8 mg/kg bw, either diluted (370 kBq/kg bw) or undiluted (1.95 GBq/kg bw) (Burka, Washburn & Irwin, 1991).

In vitro incubation of rat liver microsomes with [^{14}C]furan in the presence of NADPH resulted in the covalent binding of furan-derived radioactivity to microsomal protein. Compared with microsomes from untreated rats, a 2- to 3-fold increase in binding was observed with microsomes from PB-treated rats, and a 4- to 5-fold increase was observed with microsomes from rats pretreated with imidazole or PY. Covalent binding was reduced with microsomes from rats pretreated with β-NF (Parmar & Burka, 1993).

In an in vitro study, an oligonucleotide synthesized with nucleosides linked via the 2′ amino group on the ribose to furan formed interchain crosslinks upon chemical oxidation (Halila et al., 2005).

(ii) BDA

In order to characterize the chemistry by which the reactive microsomal metabolite of furan, BDA, could alkylate protein, the products formed upon reaction of this unsaturated dialdehyde with *N*-acetyl-L-lysine, *N*-acetyl-L-cysteine and GSH were investigated. BDA reacted rapidly and completely with amino acid residues to form pyrrole and pyrrolin-2-one derivatives. The authors speculated that the ease with which BDA crosslinks amino acids suggests that pyrrole–thiol crosslinks may be involved in the toxicity observed following furan exposure (Chen, Hecht & Peterson, 1997).

BDA reacts in vitro with 2′-deoxynucleosides (Gingipalli & Dedon, 2001; Byrns, Predecki & Peterson, 2002). Reaction of BDA with 2′-deoxycytidine (dCyd) was greater than that with 2′-deoxyadenosine (dAdo) or 2′-deoxyguanosine (dGuo). The reaction with dCyd formed a stable oxadiazabicycloctaimine adduct

(Gingipalli & Dedon, 2001). BDA was reacted with nucleosides dCyd, dGuo and dAdo, and the reaction products that formed across a pH range of 6.0–8.0 at 37 °C were characterized. All of the nucleosides formed reasonably stable adducts with BDA. Thymidine did not react with BDA (Byrns, Predecki & Peterson, 2002).

It was found subsequently that although the adducts formed with dCyd were relatively stable, the initial adducts formed with dGuo and dAdo readily undergo dehydration to form substituted etheno adducts. This rearrangement exposes a reactive aldehyde, which has significant implications for the toxicological properties of furan, such as induction of point mutations, as has been observed with other etheno-dAdo and etheno-dGuo adducts; and the generation of DNA–DNA and/or DNA–protein crosslinks, as has been observed with a number of α,β-unsaturated aldehydes and dialdehydes (Byrns, Vu & Peterson, 2004). These investigators then developed an assay for the detection of BDA-derived DNA adducts. The dCyd and dAdo adducts were detected in digests of DNA treated with nanomole per litre concentrations of BDA as well as in DNA isolated from *Salmonella typhimurium* strain TA104 that had been treated with mutagenic concentrations of BDA used in a mutagenicity assay. There was a dose-dependent increase in the concentration of dCyd and dAdo adducts that roughly corresponded to the previously published mutagenicity results (see Peterson, Naruko & Predecki, 2000 in section 2.2.4) (Byrns et al., 2006).

To investigate the capability of BDA to induce DNA crosslinking, the alkaline elution assay for DNA strand breaks was performed. CHO cells that were labelled for 24 h with 0.9 mBq/ml [2-^{14}C]thymidine were lysed, and elution from cellulose ester filters was measured. The average retention of DNA from control cells was 0.96 ± 0.02. Cells were incubated for 90 min with BDA at 0.17, 0.5 and 1.5 mmol/l, with and without methyl methanesulfonate (MMS) at 1 mmol/l for 60 min prior to lysis to induce single-strand DNA breaks, which increase the kinetics of DNA elution. MMS reduced retention to 0.55 ± 10. BDA, like several other aldehydes, was found to reduce the effect of MMS on the kinetics of DNA elution in a dose-dependent manner, which indicates that it induced either interstrand or DNA–protein crosslinks (Marinari, Ferro & Sciaba, 1984).

Induction of crosslinking was assessed in L5178Y tk$^{+/-}$ mouse lymphoma cells using the alkaline comet assay. Gamma irradiation–induced DNA migration was not inhibited by concentrations of BDA up to 100 µmol/l, in contrast to glutaraldehyde, which produced inhibition at greater than 10 µmol/l (Kellert et al., 2008b).

In splenocytes isolated from B6C3F1 mice 5–6 weeks of age, furan administered intragastrically at 8 and 15 mg/kg bw per day, 5 days/week, for 4 weeks did not result in DNA crosslinks measured in a comet assay in which slides were irradiated with gamma rays prior to cell lysis to induce DNA breaks (data not shown) (Leopardi et al., 2010).

(b) Effects on gene expression

Male Sprague-Dawley rats were administered furan (>99% pure) intragastrically at 4 or 40 mg/kg bw in corn oil for 1, 3, 7 or 14 days. Liver was collected and frozen in liquid nitrogen, and slices were taken for histopathology. Total ribonucleic acid (RNA) was isolated, and from it, complementary DNA (cDNA)

targets were prepared by oligo(dT)-primed polymerization. Fluorescently labelled targets were hybridized to cDNA chips on which sequence-verified rat clone cDNAs were applied. Hepatocellular degeneration was observed as early as the first day in all high-dose rats and was present in all animals at this dose at the 3- and 7-day time points and in one animal at the 14-day time point. Inflammation occurred in both dose groups at all time points. Biliary hyperplasia and cholangiofibrosis appeared by day 7 in the high dose group.

Principal component analysis of gene expression was suggestive of dose- and time-dependent responses in alterations. Focus was placed on genes altered at later time points when cholangiofibrosis was present. Mild to subtle alterations were studied, as it was considered that a subset of these could represent diluted gene expression originating in minority cell types such as bile duct cells. Among such slightly elevated gene expressions were those of *O*-acetyl disialoganglioside synthase and stellate cell activation-associated protein, whose products could contribute to fibrosis. The gene expression changes were assumed to be indicative of alterations in transcription, but changes in cell types present could contribute. Similar gene expression changes were found in different lobes of the liver, but the magnitude of induction/repression was higher in the right lobe, followed by the caudate (Hamadeh et al., 2004).

Liver was obtained from the F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mgkg bw per day) in the Gill et al. (2009) 90-day intragastric study (section 2.2.2) for study of gene expression. Total RNA was isolated, and cDNA was generated. In high-dose males, CYP1A1 and GST-P transcript levels were increased, whereas other CYPs were reduced. Females at this dose had increased expression of CYP3A11, GST-P, cyclin D1, p21 and cOX2, whereas some CYPs were reduced. Proliferating cell nuclear antigen expression was increased in males at 0.03 mg/kg bw and in females at 0.12 mgkg bw, and p21 was increased in both at the low dose. Thus, a no-observed-effect level (NOEL) was not established (Curran et al., 2009).

(c) Gene mutations in induced neoplasms

Liver tumours induced in infant male B6C3F1 mice by preweaning administration of furan, either as a single dose of 400 mg/kg bw or as six doses of 200 mg/kg bw, were collected for analysis of Ha-*ras*-1 (*Hras1*) mutations. After polymerase chain reaction (PCR) amplification of isolated DNA, slot-blot oligonucleotide hybridization was used to screen for mutations at known mutational hot-spots in the first three exons of *Hras1*. The relative frequency of *Hras1* activation was found in 82% of the 28 tumours analysed from the single-dose group and in 32% of the 28 tumours analysed from the multiple-dose group (Johansson et al., 1997).

(d) Hepatotoxicity

(i) Mice

Female B6C3F1 mice were dosed for 3 weeks under the NTP (1993) bioassay conditions (i.e. intragastric administration for 5 days/week) with either vehicle (corn oil) or furan (containing 0.025% butylated hydroxytoluene [BHT] as a

polymerization inhibitor) at doses of 4, 8 or 15 mg/kg bw per day. In a second experiment, female mice were given corn oil, furan at 15 mg/kg bw per day or furan at 15 mg/kg bw per day plus intraperitoneal injections of ABT, an inhibitor of CYP, at 10 mg/kg bw per day, 7 days/week, starting 5 days prior to the start of treatment with furan. Control and furan-treated groups consisted of 6–11 animals. Seven days prior to necropsy, mice were implanted with mini-osmotic pumps to dispense 5-bromo-2′-deoxyuridine (BrdU) for incorporation into replicating cells. Immediately prior to sacrifice, blood samples were collected for determination of ALT, sorbitol dehydrogenase (SDH) and total bile acids. At necropsy, the liver was excised, weighed and examined for visible lesions. Representative liver samples were taken from each liver lobe and prepared for histopathological evaluation, measurement of apoptosis and immunohistochemical measurement for determination of BrdU LI. As no significant differences were observed between the lobes of control and furan-dosed mice, measurements of labelling and apoptotic indices were based on the median lobe.

There was no evidence of clinical signs, mortality or changes in body weight related to dosing of furan. Whereas liver weight was not affected by furan dosing in the first experiment, in the second experiment, liver weight was slightly increased (by about 1.2-fold) compared with controls in mice dosed with furan at 15 mg/kg bw per day, both with and without ABT. The serum activities of both ALT and SDH were increased by furan dosing at 8 and 15 mg/kg bw per day, and concentrations of serum total bile acids were statistically significantly increased at 15 mg/kg bw per day. The only pathological finding in liver associated with furan dosing was a dose-related increase in minor subcapsular inflammation with minimal histological evidence of occasional necrosis in mice receiving 8 and 15 mg/kg bw per day. Co-treatment with ABT counteracted the observed furan-induced inflammation and necrosis as well as the elevation in serum ALT, SDH and total bile acids. Mice exposed to ABT displayed minimal to mild hepatocyte vacuolization, which was compatible with lipid accumulation; this observation was absent from control mice or mice that received furan alone. The apoptotic index determined from morphological evaluation of apoptotic cells and bodies in H&E-stained liver sections from the first experiment was statistically significantly increased above control values in the 8 and 15 mg/kg bw per day groups from the first experiment (6- and 15-fold, respectively) and in the 15 mg/kg bw per day group from the second experiment (approximately 8-fold). A significant dose-dependent increase in the hepatocyte LI relative to control values was noted at all dose levels of furan in the first experiment (1.3-, 1.6- and 1.7-fold at doses of 4, 8 and 15 mg/kg bw per day, respectively) and in the mice dosed with furan alone at 15 mg/kg bw per day in the second experiment (1.4-fold). Concurrent exposure to ABT statistically significantly reduced the effects of furan at 15 mg/kg bw per day on labelling and apoptotic indices—in the case of LI, significantly below control values (Fransson-Steen et al., 1997).

Groups of 15 female B6C3F1 mice were dosed intragastrically with furan (>99% pure) in corn oil at doses of 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, 5 days/week, for 3 weeks. Blood samples were collected at sacrifice for determination of ALT activity. For quantification of LI of hepatocytes in the left lobe

of the liver, 10 mice at each dose level received BrdU in the drinking-water for 5 days prior to sacrifice. Complete macroscopic examinations were conducted at necropsy. The liver was removed for organ weight determination, and representative sections of the left, median, right posterior, right anterior and caudate lobes of the liver and sections of macroscopic lesions were prepared using H&E stain and evaluated histologically for the presence of liver cytotoxicity. For LI analysis, a section of liver was processed for immunohistochemical assessment of BrdU incorporation. All animals survived to terminal sacrifice, and no dosing-related clinical observations relevant to liver were noted. Dosing with furan for 3 weeks had no effect on body weight or body weight gain. Doses of 1.0 mg/kg bw per day and higher resulted in a dose-related increase in ALT activity. Relative liver weights and LI were increased at the highest dose, 8.0 mg/kg bw per day, compared with untreated controls. An increased incidence of hepatic cytotoxicity, characterized by hepatic parenchymal degeneration and inflammation and/or subcapsular hepatocyte necrosis and inflammation localized on visceral surfaces of the liver in contact with the forestomach, was noted at doses of 1.0 mg/kg bw per day and higher. A histopathological qualitative assessment of hepatocyte proliferation was performed based on increases in mitotic figures, which were noted in 1/10, 3/10, 4/10 and 5/10 mice in 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day dosed groups, respectively (Moser et al., 2009).

Male B6C3F1/CrlBR mice, six per group, were dosed intragastrically 5 days/week with furan in corn oil (purity >99%) at 15 mg/kg bw per day, the highest dose used in the NTP (1993) 2-year assay. The animals were sacrificed after 1, 3 or 6 weeks of dosing with furan or after 1 or 6 weeks receiving the corn oil vehicle alone. Six days prior to sacrifice, osmotic pumps containing [^{3}H]thymidine were implanted subcutaneously for determination of LI. At sacrifice, the livers were collected and sections prepared for histoautoradiographic determination of LI in hepatocyte nuclei and histopathological evaluation. After 1 week of administration of a dose of 15 mg/kg bw per day, 5 days/week, only an occasional necrotic cell or small cluster of inflammatory cells was noted. In some animals, the visceral surface of the liver was irregular, with small foci of necrotic cells and early fibrosis. At the 3-week sacrifice, the subcapsular parenchyma of the visceral surface had necrosis and inflammation that was more extensive than at 1 week. Otherwise, the liver was normal with the exception of some perinuclear vacuolization of the cytoplasm. At the 6-week sacrifice, a centrilobular pattern of hepatocyte vacuolization was evident. No bile duct proliferation was noted. A marked increase in hepatocyte LI was noted at week 1, which decreased linearly through 6 weeks (LIs of 25.1, 12.0 and 3.2 for weeks 1, 3 and 6, respectively, compared with 0.41 and 0.89 for controls at 1 and 6 weeks, respectively). Although the proliferative response decreased over the 6 weeks, it was still significantly elevated over control values (Wilson et al., 1992).

(ii) Rats

To investigate the early cellular changes in liver associated with furan-induced cholangiocarcinogenesis, young adult male F344 rats were dosed intragastrically with furan (>99% pure) in corn oil once a day, 5 days/week, for 2–3 weeks at doses of 0, 15, 30, 45 and 60 mg/kg bw per day. During the course of the

dosing period, rats receiving furan exhibited a dose-dependent decrease in their average body weight gain. Survival was decreased at 45 mg/kg bw per day, and all rats dosed with 60 mg/kg bw per day were dead by the end of the second week. The most conspicuous feature observed in the livers of animals dosed with 30 mg/kg bw per day and greater was a cholangiofibrosis characterized by the presence of bile ductular hyperplasia, intestinal metaplasia and fibrosis. This lesion was found to be almost exclusively localized to the caudate liver lobe, whereas the other lobes primarily showed cirrhosis. Both the hyperplastic bile ductular epithelial cells and the intestinal-like epithelial cells in the cholangiofibrotic areas selectively exhibited a strongly positive immunohistochemical staining for cytokeratin 19, normally found in intrahepatic biliary epithelial cells. However, in contrast to the hyperplastic bile ductules, electron microscopy of the metaplastic intestinal glands revealed them to be composed mostly of columnar epithelial cells. A precursor relationship between the bile ductular structures and the metaplastic cholangiofibrotic areas was proposed (Elmore & Sirica, 1991).

Male and female F344/CrIBR rats, six per group, were dosed intragastrically 5 days/week with furan (purity >99%) in corn oil at 8 mg/kg bw per day, the highest dose used in the NTP (1993) assay. The animals were sacrificed after 1, 3 or 6 weeks of dosing with furan or after 1 or 6 weeks receiving the corn oil vehicle alone. Six days prior to sacrifice, osmotic pumps containing [^{3}H]thymidine were implanted subcutaneously for determination of LI. Immediately prior to sacrifice, a blood sample was collected for determination of AST, ALT and LDH. At sacrifice, the livers were collected and sections prepared for histoautoradiographic determination of LI in hepatocyte nuclei and histopathological evaluation. After 1 week of administration of furan at 8 mg/kg bw per day, 5 days/week, subcapsular focal areas of inflammatory cell infiltrate were observed on the visceral surface of the liver. Several small foci of inflammatory cells were found scattered randomly in the hepatic parenchyma. While centrilobular hepatocytes were vacuolated, the periportal cells were hypereosinophilic. At the 3- and 6-week sacrifices, a prominent pattern of visceral surface lesions and hypereosinophilic periportal hepatocytes was evident. Bile duct hyperplasia accompanied by peribiliary fibrosis was observed in the liver parenchyma. In some cases, inflammatory cells from the subcapsular visceral surface lesion extended into the parenchyma, where they surrounded a small number of degenerated and/or necrotic hepatocytes. The subcapsular inflammation was often associated with a light brown pigment in macrophages within the inflammatory zone. Mild to moderate bile duct proliferation within this zone was evident in some sections. By 6 weeks of furan dosing, cholangiofibrosis was detected in subcapsular areas of the left or caudate lobes of the liver. Signs of metaplasia localized within these cholangiofibrotic areas were observed in two rats as ducts composed of columnar cells with basal nuclei. These cells resembled intestinal cells and have been referred to variously as intestinal cell metaplasia, bile duct hyperplasia and cholangiofibrosis (Elmore & Sirica, 1991) (see section 2.2.2). Evaluation of these ducts in liver sections that had been processed for autoradiography revealed that often the nuclei of ductal cells incorporated thymidine and were therefore actively dividing. This surface lesion was qualitatively similar to, but less severe and less advanced than, the cholangiohepatitis described in a 90-day gavage study with 30 mg/kg bw per day in male F344 rats (NTP, 1993).

Hepatocyte proliferation in the livers of male and female rats was significantly elevated above control values at all time points examined. In furan-dosed males, the LI was 3.2, 9.2 and 6.5 at weeks 1, 3 and 6, respectively, compared with control values of 0.08 and 0.29 at weeks 1 and 6, respectively. In the dosed females, the LI was 11.7, 9.2 and 14.4 at weeks 1, 3 and 6, respectively, compared with control values of 0.77 and 0.75 at weeks 1 and 6, respectively. No significant increases in plasma enzyme levels were observed (Wilson et al., 1992).

To determine if the hepatic tumours induced within rat liver after long-term furan dosing could be correlated in terms of both their cellular composition and their liver lobe sites of origin with the small-intestinal metaplasia and cholangiofibrosis that occurs early and essentially within the right and caudate liver lobes of rats following short-term chronic exposures to furan (Elmore & Sirica, 1991), two separate experiments were performed in young adult male F344 rats weighing 160–190 g. In the first experiment, 12 rats were dosed intragastrically with furan (>99% pure) in corn oil at 30 mg/kg bw per day, 5 days/week, for 13 weeks. In the second experiment, three groups of 10 rats each were dosed with furan at 30 mg/kg bw per day, as above, for 6, 9 or 12 weeks. In both experiments, the rats were sacrificed at 16 months after initiation of the furan dosing. Complete necropsies were performed on each rat, with particular attention being paid to the liver lobe distribution of the hepatic tumours. Samples of all resulting hepatic and non-hepatic tumours as well as random non-tumorous portions of each liver lobe were processed for histopathology.

The furan-dosed rats in both experiments exhibited very high incidences of hepatic adenocarcinoma at 16 months. In the first experiment, this incidence was 90%, and in the second experiment, it was 75% and 71% for rats that received furan for 9 and 12 weeks, respectively. Even when furan was administered for only 6 weeks, 44% of the dosed rats were found to have a hepatic adenocarcinoma at the end of the 16-month experimental period. The hepatic adenocarcinomas that developed in the furan-dosed rats were preferentially localized to the right/caudate liver lobes. The epithelial glandular structures within the adenocarcinomas were characterized by their abundant mucin production. These glandular structures were also found to exhibit a homogeneous pattern of staining for cytokeratin 19, similar to the early developing cholangiofibrosis (Elmore & Sirica, 1991). Twenty-six of the twenty-seven hepatic adenocarcinomas exhibited evidence of small-intestinal differentiation, as reflected by the presence of goblet cells, Paneth cells and serotonin-positive neuroendocrine cells. The authors concluded that the small-intestinal metaplasia and subsequent cholangiofibrosis developing early in the right/caudate liver lobes of furan-dosed rats do not simply reflect reactive changes, but strongly correlate with the high incidences of intestinal-type primary hepatic adenocarcinoma that occurs in the right/caudate liver lobes of rats after long-term dosing with furan (Elmore & Sirica, 1993).

Male Sprague-Dawley rats 6–8 weeks of age were dosed intragastrically with furan (>99% pure) at 30 mg/kg bw per day, 5 days/week, for periods up to 3 months. Animals ($n = 5$) were removed at periodic intervals beginning 8 h after the first furan dose was administered, with the caudate, left, right and median liver lobes processed for histopathological analysis. In addition, livers from at least three

animals per time point were assessed by immunohistochemistry for a variety of markers related to cellular proliferation, apoptosis, cell communication and DNA oxidative damage. Tissue samples in which cholangiofibrosis was evident (caudate and right liver lobes) were also processed for gene expression analysis (messenger ribonucleic acid [mRNA] quantification) by real-time PCR.

Within 8 h after the initial furan dosing, hepatocyte necrosis and apoptosis were evident in most liver lobes, whereas after 3 days of furan dosing, hepatocytes within the parenchymal region were mostly restored by compensatory hepatocyte proliferation. By 7 days of furan exposure, focal proliferation of biliary ducts into areas of hepatocellular damage, with no or limited compensatory repair, was evident, which correlated with the subsequent incidence and location of cholangiofibrosis (by day 12). Hepatic areas of significant CYP2E1 expression (centrilobular), as determined by immunostaining, also correlated with furan-induced cellular necrosis and apoptosis. Furan dosing also caused a significant increase in 8-oxo-2′-deoxyguanine in necrotic centilobular and subcapsular liver areas. At the end of the dosing period or after 1 month of recovery, increased staining for 8-oxo-2′-deoxyguanine was still evident in cholangiofibrotic lesions as well as in hepatocytes near focal areas of inflammation. Gene expression was assessed in the cholangiofibrotic lesions that persisted 1 month after termination of dosing. In total, 135 genes were determined to be upregulated (greater than 2-fold increase compared with controls), including a number of genes associated with cellular/oxidative stress, transformation, growth arrest, DNA damage and proliferation. Cholangiofibrotic lesions, associated with a marked inflammatory reaction, were found to persist and continue to expand following 1 month after termination of furan dosing. The authors suggested that chronic indirect DNA damage from oxygen radicals, as evidenced by DNA oxidation and the continued expression of genes associated with oxidative stress in areas of cholangiofibrosis, ultimately contributes to the progression and development of cholangiocarcinomas (Hickling et al., 2010a).

Additional analysis of liver and cholangiofibrotic areas was conducted at the same time intervals as previously described (Hickling et al., 2010a) for identification of fibroblast populations (hepatic, vascular and biliary) and the determination of various growth factors and cell-specific markers (i.e. epithelia, dendritic cells, leukocytes). Following furan-induced centrilobular parenchymal damage and subsequent compensatory repair, proliferation of ductular biliary cells was also evident, extending beyond the portal tracts into the liver parenchyma. Altered differentiation of the biliary epithelium into cells expressing a hepatocyte phenotype was also observed. In areas of more extensive damage, however, cells with an intestinal metaplastic phenotype also developed following 12 days of furan dosing. These cells exhibited increased expression of the mesenchymal-epithelial transition growth factor and proliferating cell nuclear antigen, markers for active cellular proliferation and invasive growth. Reconstruction by serial sectioning of cholangio-fibrotic areas showed that these proliferating cells with an intestinal metaplasia phenotype were an integral part of the biliary ductal system. The authors proposed that this process of abnormal hepatocyte differentiation during the repair process following high-dose furan administration leads eventually to aberrant biliary duct proliferation and, eventually, cholangiofibrosis (Hickling et al., 2010b).

(e) Hormonal activity/effects

Serum and testes were obtained from the F344 rats at all dose levels (0, 0.03, 0.12, 0.5, 2.0 and 8.0 mg/kg bw per day) in the Gill et al. (2009) 90-day intragastric study (section 2.2.2) for analysis of the following parameters: serum testosterone levels, intratesticular testosterone levels, serum luteinizing hormone (LH), mRNA expression of the testicular LH receptor, testicular cholesterol side-chain cleavage enzyme, testicular benzodiazepine receptor and testicular steroidogenic acute regulatory (StAR) protein levels. Serum testosterone levels increased with dose, achieving statistical significance at 2.0 and 8.0 mg/kg bw per day (elevations of 2.5- and 3-fold, respectively, from control). All doses of furan resulted in significantly increased intratesticular testosterone levels. Significantly decreased LH levels were observed in rats dosed with furan at 2.0 and 8.0 mg/kg bw per day. The mRNA expression levels for the LH receptor, cholesterol side-chain cleavage enzyme and peripheral dibenzodiazepine receptor in rat testes preparations were not significantly affected by furan dosing. In contrast, the mRNA expression levels of StAR protein were significantly increased following furan dosing, with all doses resulting in significant increases. This study indicates that furan does not disrupt testosterone synthesis by affecting the rate of its formation from cholesterol or by altering sensitivity of the testicular LH receptor. As the functional capacity of Leydig cells to produce testosterone was not compromised, the biological significance of the changes observed remains uncertain (Cooke, 2009).

2.3 Observations in humans

A search for pertinent publications on clinical observations and epidemiological studies of the potential health effects of exposure to furan was conducted using various search engines to query multiple databases, including PubMed, Google, Wiley Interscience, Elsevier and SpringerLink.com. No relevant publications were identified, consistent with the observation of Heppner & Schlatter (2007), who noted, in their review of furan, that “there are no data from human studies.”

2.3.1 Biomarker studies

Plasma and urine samples were obtained from 100 healthy volunteers (49 men and 51 women) between the ages of 30 and 70 following a 12 h fast. The participants consumed a normal Korean diet and did not regularly consume large amounts of furan-containing food items, listed by the authors as smoked salmon, powdered milk, apple juice, spaghetti, potatoes, peas, coffee or canned or jarred food. The length and timing of the collection period for urine were not indicated. Blood samples were analysed for concentrations of glucose, total, high-density and low-density lipoprotein cholesterol, triglycerides and enzyme activities of ALT, AST and gamma-glutamyl transpeptidase (GGT). Unmetabolized furan was detected in urine samples from 56 of the participants (31 males, 25 females). Of these, only 15 samples exceeded the limit of quantification (LOQ) of 1.0 µg/l, up to the highest value of 3.14 µg/l. The authors did not indicate how the data from samples that were at the LOQ were handled. Unchanged furan was not detected in blood samples.

The investigators found a strong positive correlation ($r = 0.56$, $P < 0.0001$) between the activity of GGT and urinary furan concentration in individuals with detectable urinary furan (Jun et al., 2008).

3. ANALYTICAL METHODS

3.1 Chemistry

Furan (C_4H_4O) (CAS No. 110-00-9) is a colourless, flammable liquid with an ethereal odour, having a low molecular weight of 68.08, a high volatility, with a boiling point of 31.36 °C, and a specific gravity of 0.9371 at 101.1 kPa. Furan is insoluble in water but is soluble in alcohol, ether and most common organic solvents. Chemically, furan is classified as a cyclic, dienic ether (NTP, 1993).

All ring atoms of furan lie in a plane and form a slightly distorted pentagon. The small dipole moment of furan (0.71 D) confirms that one electron pair of the oxygen atom is included in the conjugated system and therefore delocalized. Data obtained by ultraviolet (UV) and nuclear magnetic resonance (NMR) indicate that a diamagnetic ring current is induced in the furan molecule, which fulfils an important experimental criterion for aromaticity. Furan undergoes reactions with electrophilic reagents, often with substitution. However, it can also react by addition and/or ring opening, depending on reagents and reaction conditions (Eicher, Hauptmann & Speicher, 2003). Furan is stable to alkalis but forms resins on evaporation or in contact with mineral acids. Upon standing, furan turns brown; however, the colour change can be retarded with the addition of a small amount of water. Unless stabilized, furan will react slowly with air to form an unstable, explosion-prone peroxide (HSDB, 2001).

3.2 Description of analytical methods

3.2.1 Introduction

Analytical methods for the determination of furan in foods have been reviewed by some authors (Crews & Castle, 2007a,b; Wenzl, Lachenmeier & Gökmen, 2007; Wenzl, 2008; Vranová & Ciesarová, 2009). The extraction strategy and the measurement techniques have been defined taking into account the high volatility and the low molecular weight of the analyte.

3.2.2 Quantitative methods

(a) Sample preparation

To avoid furan loss, food samples and standards need to be chilled and handled quickly (Crews & Castle, 2007a). Temperatures between 0 °C and 10 °C have been applied, but most laboratories store samples at 4 °C for at least 4 h before homogenization. In the case of frozen samples, the thawing should be performed under controlled conditions at low temperature (e.g. in an ice bath), and increased attention has to be paid to sample homogeneity after thawing (Wenzl, 2008).

Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples (non-homogeneous and with variable amounts of fat) should be homogenized prior to extraction (Wenzl, Lachenmeier & Gökmen, 2007). Homogenization of solid samples has to be done as quickly as possible; depending on the physical state of the sample and other specific properties, such as the fat content, different strategies for sample homogenization can be applied (USFDA, 2004; Nyman et al., 2008). Samples that formed a starch gel can be treated in the sealed HS vial with amylase at room temperature overnight for about 14 h (Zoller, Sager & Reinhard, 2007). During homogenization and subsampling, it is recommended that the samples be kept in an ice bath or that dry ice be added in order to avoid furan losses. For homogenization, blenders and Ultra-Turrax are most frequently employed. In general, test portion size varies from 0.05 to 10 g, depending on the physical state of the samples and on the furan content.

(b) Addition of water or saline solutions

Water is usually added to the samples in order to reduce the viscosity, which favours equilibration and changes the ratio between the gas phase and sample phase (Wenzl, 2008). The amount of added water varies from 0.5 to 15 ml. In the procedure described by the United States Food and Drug Administration (USFDA), water was replaced by a saturated sodium chloride solution (USFDA, 2004), whereas in other methods, the salt is added directly into the vial (Becalski et al., 2005; Goldmann et al., 2005; Altaki, Santos & Galceran, 2007; Yoshida et al., 2007; Zoller, Sager & Reinhard, 2007; Altaki, Santos & Galceran, 2009; Bononi & Tateo, 2009; Jestoi et al., 2009). The use of salt (sodium chloride, sodium sulfate or ammonium sulfate) might reduce the solubility of furan in the aqueous phase and hence lead to more intensive signals. However, the relative signal intensities of furan and internal standard did not significantly change (Crews et al., 2007; Wenzl, 2008).

(c) Addition of internal standard

All published methods use an internal standard in furan analysis. Most laboratories use deuterium-labelled furan ([$^2H_4$]furan), d_4-furan, which is normally added to the homogenized sample before the extraction. Only one method describes the addition of d_4-furan at the homogenization step (Becalski et al., 2005). However, the authors noted a significant variation in the recovery of the samples, which may impart errors of a 10–20% magnitude in the determination of furan. It is recommended that the content of d_4-furan be adjusted to the content of native furan in the test sample. Senyuva & Gökmen (2005) reported the only method in which d_6-acetone was used as internal standard. According to the authors, complete resolution of the peaks of d_4-furan and furan could not be achieved under the gas chromatographic (GC) separation conditions. Instead, d_6-acetone could be completely resolved from the native furan and was found to be more suitable than d_4-furan as an internal standard.

(d) Headspace (HS) extraction

The first quantitative method for furan analysis in different matrices employed HS extraction (USFDA, 2004), and several procedures described in the literature are based on this approach, with or without modifications. Both static and dynamic HS extractions have been used (Hoenicke et al., 2004; Becalski & Seaman, 2005; Becalski et al., 2005; Kuballa, Stefan & Nina, 2005; Senyuva & Gökmen, 2005; Hasnip, Crews & Castle, 2006; Nyman et al., 2006; Crews et al., 2007; Senyuva & Gökmen, 2007; Vranová, Bednáiková & Ciesarová, 2007; Yoshida et al., 2007; Zoller, Sager & Reinhard, 2007; Morehouse et al., 2008; Nyman et al., 2008; Roberts et al., 2008; Lachenmeier, Reusch & Kuballa, 2009).

Static HS extraction is a relatively simple and well-proven methodology very suitable to volatile compounds. In this technique, a food sample in liquid or slurry form is heated in a sealed vial to achieve equilibrium partitioning between the liquid phase and the gaseous HS. The HS gas is sampled and the vapour injected into a GC (Vranová & Ciesarová, 2009). In dynamic HS extraction (purge and trap), the volatiles are removed by bubbling a purge gas through the sample matrix and subsequently collected on an analytical trap. After the purging is complete, the trap is heated, and the volatiles are released and delivered to a GC for separation and detection (Slack, Snow & Kou, 2003). The direct and accurate analysis of volatiles in foods by HS extraction requires careful standardization of parameters such as equilibration time and temperature. According to published methods, the equilibration temperature in HS analysis varies between 30 °C and 80 °C, and the equilibration time varies between 10 and 60 min, but most methods use 30 min. Prolonged equilibration time in static HS extraction can be reduced by using automated vial shaking, as suggested by Hasnip, Crews & Castle (2006).

(i) Trueness of the HS extraction methods

Partitioning of analytes for HS sampling can be increased by raising the incubation temperature; for many analytes, it is typical to use a temperature of about 80 °C (Crews & Castle, 2007b). However, some authors have noted that furan can be formed in certain matrices when the equilibration temperature is above 60 °C, especially in green coffee (Senyuva & Gökmen, 2005; Hasnip, Crews & Castle, 2006; Nyman et al., 2008), although others have observed furan formation only above 80 °C (Nyman et al., 2006; Yoshida et al., 2007; Morehouse et al., 2008). The method initially described by the USFDA employed an equilibration temperature of 80 °C, which was changed to 60 °C after these observations (USFDA, 2004). The reduction in the incubation temperature raised questions regarding the accuracy of previously reported data for canned and jarred foods that were determined using 80 °C and posted on the USFDA web site (Nyman et al., 2008). Therefore, additional HS studies were conducted to address this concern by analysing selected foods containing low levels of furan and coffee by the old and new methods. The furan level found in green beans increased by 3 ng/g (24%) between 60 °C and 80 °C (not significantly different at the 5% level), whereas in other foods, no significant increase was noted, which indicates that the data previously published are valid (Nyman et al., 2008). According to Senyuva & Gökmen (2005), a matrix-matched calibration for each particular food matrix is necessary to compensate for furan formation during HS sampling and thus to quantify furan more accurately.

(e) Headspace solid-phase microextraction (HS-SPME)

Several methods described in the literature have used the headspace solid-phase microextraction (HS-SPME) technique for furan determination in different matrices (Fan, 2005a,b; Goldmann et al., 2005; Ho, Yoo & Tefera, 2005; Bianchi et al., 2006; Fan & Mastovska, 2006; Fan & Sommers, 2006; Altaki, Santor & Galceran, 2007; Fan & Geveke, 2007; Limacher et al., 2007; Fan & Sokorai, 2008; Fan, Huang & Sokorai, 2008; Limacher et al., 2008; Altaki, Santos & Galceran, 2009; Bononi & Tateo, 2009; Jestoi et al., 2009; Kim et al., 2009a,b; La Pera et al., 2009). SPME is an alternative solvent-free sampling technique widely used for the analysis of volatile compounds (Wenzl, Lachenmeier & Gökmen, 2007). In SPME, a fibre coated with a polymeric material is first exposed to the HS vapours of the vial to absorb volatiles. Then, the fibre is thermally desorbed in the injection port of the GC to drive off the volatiles onto the GC column (Crews & Castle, 2007a). Proper selection of the SPME fibre is important to increase the extraction yield (Wenzl, Lachenmeier & Gökmen, 2007). According to published methods, the most used fibre for furan analysis is the 75 μm carboxen–polydimethylsiloxane. The bipolar phase has unique characteristics, such as pore size, distribution, volume, shape and particle size, which are ideal for small analytes (Shirey, 1999). Few methods have reported the use of other fibres, such as 85 μm carboxen–polydimethylsiloxane (Fan & Sommers, 2006; Fan & Geveke, 2007; Fan, Huang & Sokorai, 2008) and 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (Bononi & Tateo, 2009).

Extraction temperatures reported in the literature usually vary between 25 °C and 50 °C. Higher temperatures have been associated with less intense signals due to the desorption of furan from the fibre and the increase in the distribution constant of furan between the HS and the fibre coating (Bianchi et al., 2006; Altaki, Santos & Galceran, 2007). Extraction times between 10 and 30 min as well as magnetic stirring have normally been employed. Desorption has been carried out from 220 °C for 1 min up to 300 °C for 10 min.

(f) HS versus HS-SPME

In 2008, the first proficiency test on the determination of furan in baby food consisting of carrot and potato purée was conducted (Kubiak, Karasek & Wenzl, 2008). From 22 laboratory participants, about 70% applied HS coupled with gas chromatography–mass spectrometry (GC-MS), and 30% used HS-SPME with GC-MS. The results indicated that 16 out of 22 laboratories were capable of analysing furan in food matrices, reporting results that were satisfactory according to international guidelines. In relation to the performance of analytical methods, both techniques (HS and HS-SPME) were suitable for furan analysis in foods. More recently, several food samples, such as apple juice, honey, coffee, chicken pap baby food and cooked chickpeas, were analysed by automated HS and HS-SPME, both coupled to GC-MS (Altaki, Santos & Galceran, 2009). Both methods gave similar results for furan determination in selected food samples, although slightly worse precision (relative standard deviation 9–12%) and higher limits of detection (LODs) (5–20 times higher) were obtained by the HS method.

(g) Other extraction techniques used for furan analysis

(i) Solid-phase dynamic extraction (SPDE/GC-MS)

Ridgway, Lalljie & Smith (2006) used a commercial in-tube sorptive extraction device, known as solid-phase dynamic extraction (SPDE), for the extraction of furan from aqueous solutions in both HS and liquid injection modes. This technology allows the dynamic extraction of samples due to the fact that it uses significantly high amounts of sorbent material (4.5 µl) (Chromtech, 2006). The authors reported an LOD of 1.5 ng/g and observed no improvement in sensitivity compared with static HS extraction.

(ii) Microdistillation-GC-MS

Kuballa & Ruge (2005) published a comparison of the USFDA method (HS-GC-MS) and microdistillation-GC-MS. By this method, six HS vials were distilled using an automated microdistiller into prepared vials with a cooled solution. An aliquot from each solution was sampled and injected into a GC-MS system. The authors declared that this method can shorten the total time of analysis (in comparison with the USFDA method) by parallel distillation of six samples. Furthermore, the analysis of complex matrices (e.g. coffee) is easier.

(h) Determination by GC-MS

(i) Chromatographic performance

Chromatographic separation of furan from co-extractives has been mostly performed on porous layer open tubular capillary columns under a variety of instrument parameters (Crews & Castle, 2007a; Wenzl, Lachenmeier & Gökmen, 2007; Wenzl, 2008). Porous layer open tubular columns have a bonded porous polymer based on polystyrene and divinylbenzene, which is relatively inert and stable to water (Crews & Castle, 2007b). Other stationary phases have also been described for furan analysis, such as wax-based columns (polyethylene glycol) (Bianchi et al., 2006; Limacher et al., 2007; Yoshida et al., 2007; Bononi & Tateo, 2009), (5% phenyl)-methylpolysiloxane (Fan, 2005a,b; Fan & Mastovska, 2006; Fan & Sommers, 2006; Fan & Geveke, 2007; Fan & Sokorai, 2008; Fan, Huang & Sokorai, 2008) and cyanopropylphenyl polysilphenylene-siloxane (Altaki, Santos & Galceran, 2007; La Pera et al., 2009). Splitless injection, with or without cryogenically refocusing the injected HS gas, is the natural choice to obtain sufficient sensitivity of the method (Wenzl, Lachenmeier & Gökmen, 2007).

(ii) Detection

Quadrupole mass spectrometers are used in most cases to detect furan and d_4-furan in samples. Few procedures have described the use of ion trap analysers (Altaki, Santos & Galceran, 2007, 2009; Zoller, Sager & Reinhard, 2007). The positive electron ionization mode is usually applied, with 70 eV of electron energy, and the mass spectrometers are commonly operated in selected ion monitoring mode.

(iii) Confidence

The identification of furan is assured by checking for the correct retention time and for the presence and relative abundance of characteristic ions. The mass spectrum of furan has a relatively intense molecular ion with mass-to-charge ratio (*m/z*) of 68, which is used as quantifier ion, and a fragment ion *m/z* of 39 with sufficient abundance for confirmatory purposes (qualifier ion). Goldmann et al. (2005) used two characteristic ions as qualifiers, *m/z* 39 and *m/z* 69. For d_4-furan, *m/z* 72 (quantifier ion) and *m/z* 42 (qualifier ion) are usually monitored. The relative abundance between *m/z* 68 and *m/z* 39 for the test portions should agree with the average of the response ratios for the calibration standards by ± 10%, and the retention time for the test portions should agree with the average retention times for the calibration standards by ± 2% (USFDA, 2004).

(iv) Quantitative aspects

Quantification of furan in samples has been based on standard additions or external calibration graphs, both incorporating an internal standard. Crews et al. (2007) applied both approaches to samples having low (tomato sauce), medium (tomato soup) and high (coffee powder) levels of furan. The coffee powder sample contained 59 ng/g of furan when measured by external calibration and 62 ng/g of furan by standard additions. The tomato soup sample contained 24 ng/g of furan by external calibration and 28 ng/g of furan by standard additions. The tomato sauce sample contained 3 ng/g of furan by external calibration and 2 ng/g of furan by standard additions. These results were considered essentially identical, providing further evidence that both methods of quantification appear to be equally suitable. The external calibration procedure is especially useful when a large number of food samples are to be analysed, as it reduces the need for duplication of sample preparation (Crews et al., 2007).

Some published methods based on HS extraction have been demonstrated to be linear over concentration ranges up to 0–1000 ng/ml (Becalski et al., 2005; Yoshida et al., 2007). LODs and LOQs vary from 0.1 to 4.85 ng/g and from 0.44 to 13 ng/g, respectively. Recoveries of between 83% and 122% have been reported. In methods using HS-SPME, linearity over a concentration range up to 0–100 ng/g has already been reported in the literature (Bianchi et al., 2006). As SPME allows sample concentration and affords higher sensitivity (Crews & Castle, 2007b), LODs and LOQs have been reported from 0.001 to 1.9 ng/g and from 0.006 to 4 ng/g, respectively. Recoveries varied between 87% and 116%.

3.2.3 Summary of analytical methods

GC-MS has been shown to be the most suitable technique for the reliable detection of low levels of furan in foods. GC-MS is usually preceded by HS extraction or HS-SPME. Owing to the high volatility of furan, food samples and standards need to be chilled and handled quickly. Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples have to be homogenized. LODs and LOQs from 0.1 to 5 ng/g and from 0.4 to 13 ng/g, respectively, have been reported for methods based on HS extraction. Lower LODs and LOQs are reported for methods using HS-SPME. There is currently no certified reference material available, and few authors have reported the measurement

uncertainty of the methods, which varied between 9.5% and 29% (Goldmann et al., 2005; Jestoi et al., 2009). It is highly recommended that laboratories implement a well-documented internal quality control system and participate in an appropriate proficiency testing programme or interlaboratory comparison to authenticate the accuracy and reliability of data produced.

4. FORMATION, EFFECTS OF PROCESSING AND FATE IN FOOD

The formation of furan in foods has been discussed and reviewed by several authors (Yaylayan, 2006; Crews & Castle, 2007a; Vranová & Ciesarová, 2009). It has been demonstrated that furan can be formed from different precursors, either by thermal or by non-thermal processing.

4.1 Thermal-induced formation of furan in foods

Although the exact mechanism is not completely understood, data available in the literature indicate multiple routes of furan formation under heat treatment, such as thermal degradation or Maillard reaction of reducing sugars, alone or in the presence of amino acids, thermal degradation of certain amino acids and thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Fan, 2005b; Märk et al., 2006). The proposed pathways for thermal-induced formation of furan are summarized in Figure 1.

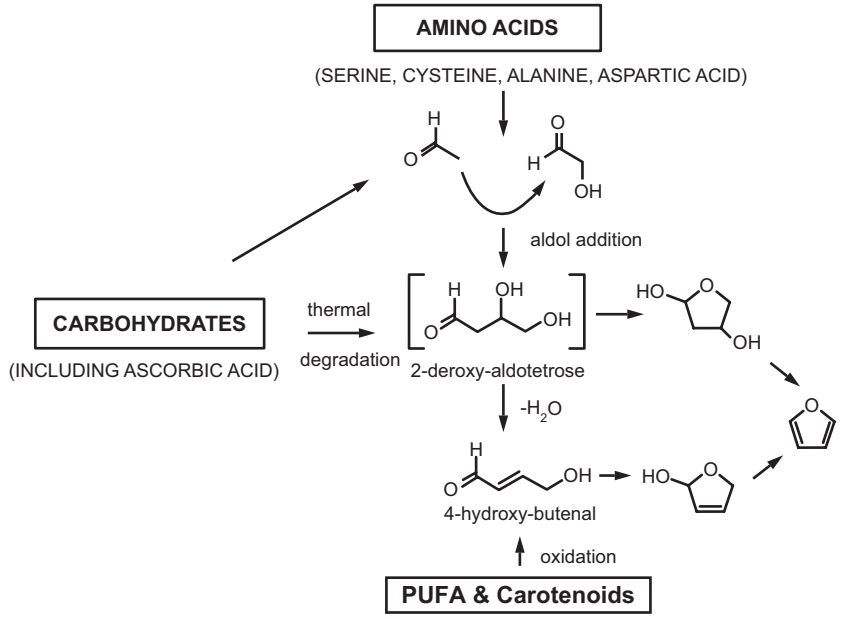
Ascorbic acid was first reported as the major precursor of furan in simple model systems (Perez Locas & Yaylayan, 2004; Märk et al., 2006). However, experiments conducted with pumpkin purée, apple cider, and carrot and orange juices have demonstrated that ascorbic acid and sugars are only minor precursors of furan in such foods (Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). Therefore, the formation of high amounts of furan may be associated with the presence of fatty acids or a combination of fatty acids with other compounds (Fan, Huang & Sokorai, 2008).

4.1.1 Chemical mechanisms

(a) Formation from carbohydrates and amino acids

The pathway of furan formation from carbohydrate degradation basically encompasses the production of aldotetrose derivatives that can undergo eventual cyclization to form furan. Reducing sugars undergo Maillard reactions in the presence of amino acids and generate reactive intermediates such as 1-deoxyosone and 3-deoxyosone. To form furan, the 1-deoxyosone has to undergo α-dicarbonyl cleavage to produce aldotetrose, followed by dehydration reactions to generate 3-furanone, which produces furan after reduction and dehydration reactions. To a lesser extent, the 3-deoxyosone undergoes α-dicarbonyl cleavage, followed by oxidation and decarboxylation to generate 2-deoxyaldotetrose, a direct precursor of furan (Perez Locas & Yaylayan, 2004).

Figure 1. Proposed pathways and precursors of furan



PUFA, polyunsaturated fatty acids

Source: Yaylayan (2006); based on Perez Locas & Yaylayan (2004) and Becalski & Seaman (2005)

(b) Formation from carbohydrates

Furan can also be formed from carbohydrates in the absence of amino acids. Hexose sugars, such as glucose, lactose and fructose, can undergo retro-aldol cleavage to generate aldotetrose, followed by dehydration to produce 3-furanone. To a lesser extent, furan can be formed from glucose after a dehydration reaction followed by a retro-aldol cleavage to form 2-deoxy-3-ketoaldotetrose. The latter intermediate can cyclize after a dehydration step to produce 3-furanone (Perez Locas & Yaylayan, 2004). Limacher et al. (2007) described a potential pathway from glucose and fructose via 4-deoxyhexo-2,3-diulose and observed that the formation mechanisms depend greatly on the reaction conditions applied, such as roasting and pressure cooking.

(c) Formation from amino acids

It has been demonstrated that amino acids capable of being metabolized to acetaldehyde and glycolaldehyde, such as serine or cysteine, can undergo thermal degradation to produce furan without the need for any other source. Acetaldehyde and glycolaldehyde react by aldol condensation, producing aldotetrose derivatives and, eventually, furan. In contrast, alanine, threonine and aspartic acid alone do not

produce furan, as these amino acids generate only acetaldehyde; therefore, the presence of reducing sugars, serine or cysteine is required to produce glycolaldehyde (Perez Locas & Yaylayan, 2004; Vranová & Ciesarová, 2009).

(d) Formation from ascorbic acid

In browning reactions, ascorbic acid behaves in a similar manner to reducing sugars (Crews & Castle, 2007a). A proposed pathway describes the oxidation of ascorbic acid to dehydroascorbic acid in aerobic conditions in food systems, followed by hydrolysis to 2,3-diketogulonic acid, which is converted to aldotetrose and later to furan. However, under anaerobic conditions, ascorbic acid cannot undergo oxidation to produce 2,3-diketogulonic acid. Instead, it can hydrolyse and undergo β-elimination followed by decarboxylation to produce 3-deoxypentosulose, which can generate 2-deoxyaldotetrose, a direct precursor of furan (Perez Locas & Yaylayan, 2004). Becalski & Seaman (2005) proposed that the formation of furan from ascorbic acid derivatives could proceed via decomposition of 2-furoic acid, a degradation product of ascorbic acid, as large amounts of furan were found in a model system formed from this compound. According to Limacher et al. (2007), furan may also be generated directly from 2-furaldehyde, which is also a degradation product of ascorbic acid, by electrophilic aromatic substitution-type reaction.

(e) Formation from polyunsaturated fatty acids

Lipid hydroperoxides can be formed non-enzymatically by reactive oxygen species or enzymatically by lipoxygenases. Subsequent homolytic cleavages of polyunsaturated fatty acid hydroperoxides, catalysed by transition metal ions, result in the formation of 2-alkenals, 4-oxo-2-alkenals and 4-hydroxy-2-alkenals. On this basis, it was proposed that the parent furan could be formed from corresponding 4-hydroxy-2-butenal through cyclization and formation of 2,5-dihydro-2-furanol and subsequent dehydration (Perez Locas & Yaylayan, 2004).

4.1.2 Formation factors

(a) Heat treatment

Several studies have demonstrated that temperature and cooking conditions are important parameters on the formation of furan. Treating sugars and organic acids in boiling water for 5 min did not result in measurable levels of furan (Fan, 2005b). In toasted bread, temperatures of about 140–160 °C resulted in a furan concentration of 3 ng/g, whereas temperatures of about 170– 200 °C resulted in furan concentrations of 12–129 ng/g (Hasnip, Crews & Castle, 2006). Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min) (Limacher et al., 2007, 2008). The link between final temperature and furan level is not clear, but generally higher temperatures produced higher levels of furan, especially above 120 °C (Hasnip, Crews & Castle, 2006; Senyuva & Gökmen, 2007). Furan can also be formed during reheating of processed foods in closed jars (Lachenmeier, Reusch & Kuballa, 2009).

(b) Precursors

(i) Carbohydrates

Studies carried out to compare the relative efficiency of different sugars to generate furan by using pyrolysis/GC-MS in conjunction with ^{13}C-labelling experiments have shown the following order of reactivity: D-erythrose > D-ribose > D-sucrose > D-glucose = D-fructose (Perez Locas & Yaylayan, 2004). Under roasting conditions, arabinose was the most efficient precursor of furan, followed by fructose, glucose and erythrose (Limacher et al., 2008).

(ii) Ascorbic acid and derivatives

The ability of ascorbic acid and its derivatives to form furan has been shown in experiments using model systems heated at 118 °C for 30 min under aqueous pressure-cooking conditions. Dehydroascorbic and isoascorbic acids generated approximately 10-fold more furan than ascorbic acid. Sodium ascorbate and isoascorbate generated less furan compared with their free acid counterparts (Becalski & Seaman, 2005). The addition of iron(III) chloride did not influence the amounts of furan formed from free acids, but significantly increased furan formation from their corresponding sodium salts (Becalski & Seaman, 2005). The furan yields from ascorbic acid were lowered in an oxygen-free atmosphere (30%) or in the presence of reducing agents (e.g. sulfite, 60%), indicating the important role of oxidation steps in the furan formation pathway (Märk et al., 2006). It has also been demonstrated that when ascorbic acid is mixed in model systems with single amino acids, sugar or unsaturated fatty acid, the mixtures produced far less furan on heating than did ascorbic acid alone (Märk et al., 2006; Limacher et al., 2007).

(iii) Polyunsaturated fatty acids

Studies performed using model systems heated at 118 °C for 30 min have indicated that only polyunsaturated fatty acids such as linoleic and linolenic acids can generate furan upon heating. It has been observed that lipids having three double bonds, such as linolenic acid, formed more furan than lipids containing two double bonds, such as linoleic acid (Becalski & Seaman, 2005; Märk et al., 2006). The influence of antioxidants (e.g. tocopherol acetate, butylated hydroxyanisole) and iron(III) chloride on furan formation from polyunsaturated fatty acids has been investigated by some authors, but no consistent results have been obtained (Becalski & Seaman, 2005; Märk et al., 2006).

(c) pH

Several studies have demonstrated that pH plays a complex role in the mechanism of furan formation (Fan, 2005b; Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). For ascorbic acid and sucrose solutions, an increase in thermally induced furan was observed at a lower pH, whereas in glucose solution and linoleic acid emulsion, the formation of furan was favoured at neutral pH. The presence of phosphate may also play a significant role in determining the level of furan, and its effect is pH dependent (Fan, Huang & Sokorai, 2008).

4.2 Non-thermal-induced formation of furan in foods

It has been reported that ionizing radiation induced the formation of furan in orange and apple juices, in grape and pineapple and in simple model systems (solutions of glucose, fructose, sucrose, ascorbic acid) (Fan, 2005a,b; Fan & Sokorai, 2008). Furan levels increased linearly as the radiation dose increased from 0 to 5 kGy and can continue to increase in the first 3 days of storage after the treatment, probably due to the residual effect of irradiation (Fan, 2005a). Compared with the thermal treatment (sterilization), an irradiation dose of 5 kGy in sugars and ascorbic acid solutions produced similar amounts of furan (Fan, 2005b). Irradiation exerts its effect in aqueous solutions through generation of free radicals from radiolysis of water. These free radicals (hydrated electrons, hydrogen atoms and hydroxyl radicals) react with the food components, such as ascorbic acid and sugars, which results in the formation of furan (Fan & Sokorai, 2008).

4.3 Effects of processing and fate in food

Limited data are available on the stability of furan during cooking, storing and reheating of meals. For coffee, the amount of furan formed in beans varies according to the level of roasting. Grinding may reduce furan levels by 10–60%, and further decreases occur in the production of instant coffee powder and in brewing (Hasnip, Crews & Castle, 2006; Zoller, Sager & Reinhard, 2007; EFSA, 2009). Losses of furan up to 85% were reported during heating of opened baby food jars over a period of 5.5 h in boiling water, whereas a reduction of ~50% was observed if the jar was opened but not heated (Goldmann et al., 2005). In vegetable purées heated in a microwave oven by different warming procedures, a decrease of 29–55% furan has been reported (Zoller, Sager & Reinhard, 2007). Reduction in the furan content of canned meats during preparation before consumption has also been reported (Kim et al., 2009a; La Pera et al., 2009).

According to some researchers, furan persists during normal warming procedures that precede consumption (Hasnip, Crews & Castle, 2006; Lachenmeier, Reusch & Kuballa, 2009). As furan appears to be well dissolved within the matrix, opening the jars exposes only a relatively small surface area. Therefore, despite furan's volatility, its evaporation is apparently hindered by its slow diffusion inside the food matrix (Lachenmeier, Reusch & Kuballa, 2009). However, if canned foods are heated in a saucepan under stirring, larger declines in furan content can be observed (Roberts et al., 2008). Losses of furan in heated foods left for cooling seem to be insignificant. No experimental data are available concerning chemical mechanisms for elimination of furan in foods.

4.4 Summary of formation and fate

Furan can be formed from different precursors either by thermal or non-thermal processing (ionizing radiation). Although the exact mechanism is not completely understood, data available in the literature indicate multiple routes of furan formation, such as thermal degradation or Maillard reaction of reducing sugars, alone or in the presence of amino acids, thermal degradation of certain amino acids, thermal oxidation of ascorbic acid, polyunsaturated fatty acids and

carotenoids and free radical reactions during irradiation (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Fan, 2005b; Märk et al., 2006). It has been demonstrated that ascorbic acid and sugars are only minor precursors of furan in foods, whereas the formation of high amounts of furan may be associated with the presence of fatty acids or a combination of fatty acids with other compounds (Limacher et al., 2007, 2008; Fan, Huang & Sokorai, 2008). Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min), and pH plays a complex role in the mechanism of furan formation. Studies on the stability of the contaminant in foods during normal warming procedures have shown conflicting results. Some authors reported furan losses of 29–85% during warming under different times (Goldmann et al., 2005; Zoller, Sager & Reinhard, 2007), whereas others have found that furan persists during normal heating practices (Hasnip, Crews & Castle, 2006; Lachenmeier, Reusch & Kuballa, 2009).

5. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

5.1 National occurrence

Furan concentration data were made available to the Committee through submissions from Australia, Brazil, Canada, the European Union (EU), Japan, the Republic of Korea, Switzerland and the USA, representing 21 countries. The total number of analytical results (single or composite samples) evaluated at the present meeting was 5662, with 58.8% from Europe, 16.7% from North America, 22.8% from Asia, 1.0% from Latin America and 0.7% from the Pacific region. The occurrence of furan has been mainly investigated in thermally processed foods, such as coffee, canned and jarred foods, including baby foods, soups and sauces. Table 13 shows the summary for individual occurrence data, including the percentage of data below the reporting limit, collected from 21 countries from 2004 to 2009.

5.1.1 Australia

Australia submitted the results of furan occurrence in different kinds of brewed coffee (FSANZ, 2009). Furan concentrations were obtained from 41 composite samples (4 samples per composite). Analyses were performed using the GC-MS technique, and the practical quantification limit (PQL) was 0.1 µg/l. All analysed samples showed furan levels above the PQL. The mean concentrations were as follows: cappuccino, 32.3 µg/l; latte, 22.9 µg/l; flat white, 33.2 µg/l; long black, 42.0 µg/l; short black, 112.5 µg/l; mocha, 23.5 µg/l; instant black, 2.0 µg/l; instant white, 2.5 µg/l; and ground coffee, 23.0 µg/l.

5.1.2 Brazil

Brazil submitted the results of furan occurrence in foods to the Committee (Arisseto, Vicente & Toledo, 2009) using the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) format. Furan concentrations were obtained from 55 individual food samples

Table 13. Summary of furan occurrence data from various countries from 2004 to 2009

Country	Number of analytical results	%	% of values below LOR[a]
Australia	41	0.7	0.0
Brazil	55	1.0	45.5
Canada	176	3.1	0.0
European Union[b]	2908	51.4	32.1
Japan	886	15.6	15.0
Republic of Korea	407	7.2	8.6
Switzerland	418	7.4	17.2
USA	771	13.6	19.7

[a] Limit of reporting (detection limit, quantification limit and practical quantification limit).
[b] Data submitted by the following European Union member states: Austria, Belgium, Cyprus, Finland, Germany, Greece, Ireland, Italy, Lithuania, the Netherlands, Poland, Slovakia, Slovenia and the United Kingdom.

purchased in 2009 from different supermarkets. Analyses were performed in samples "as bought" using the GC-MS technique. The limits of the method were, respectively, 0.7 and 2.4 µg/kg for detection and quantification. The highest average (or upper-bound mean) levels of contamination were found in roasted coffee (powder) (2998.1 µg/kg), soya sauce (25.7 µg/kg) and baby food (18.6 µg/kg).

5.1.3 Canada

Canada submitted the results of furan occurrence in foods from a recent evaluation (Becalski et al., 2009). Furan concentrations were obtained from 176 individual food samples purchased in 2006 from various retail outlets (154 canned or jarred products, including 3 coffee products, 5 packaged meat pâtés and 17 baby foods). Analyses were performed using the GC-MS technique. The LOD was 0.05 µg/kg, but only concentrations higher than 1 µg/kg were reported. The highest average levels of contamination were found in roasted coffee (powder) (4590 µg/kg), baked beans (580.6 µg/kg), instant coffee (powder) (413 µg/kg), pasta (396.4 µg/kg) and chili con carne (386.4 µg/kg).

5.1.4 European Union

The European Food Safety Authority (EFSA) submitted the results of furan occurrence to the Committee from the monitoring database of furan levels in food (EFSA, 2009), which includes data from 14 member states. Furan concentrations were obtained from 2908 samples analysed from 2004 to 2009 (124 from Austria, 229 from Belgium, 30 from Cyprus, 65 from Finland, 1746 from Germany, 48 from Greece, 264 from Ireland, 58 from Italy, 6 from Lithuania, 88 from the Netherlands,

100 from Poland, 70 from Slovakia, 35 from Slovenia and 45 from the United Kingdom). The samples were analysed without any preparation of the purchased foodstuff (e.g. coffee powder, juices, jars and cans not heated before consumption), as well as after further preparation as if consumed (e.g. brewed coffee, canned and jarred products heated before consumption). Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.1 to 40 µg/kg and from 0.2 to 100 µg/kg, respectively. The highest average levels (upper-bound mean) of contamination were found in roasted beans coffee (2272 µg/kg), roasted ground coffee (powder) (1114 µg/kg), instant coffee (powder) (589 µg/kg), baked beans (27 µg/kg), baby food (25 µg/kg) and soya sauce (25 µg/kg).

5.1.5 Japan

Japan submitted the results of furan occurrence in foods for the current meeting of the Committee from different surveys (Yoshida et al., 2007; MAFF, 2009a,b). Furan concentrations were obtained from 886 individual food samples. Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.2 to 0.5 µg/kg and from 0.4 to 2.0 µg/kg, respectively. The highest average levels of contamination were found in fermented soya bean pastes made with soya bean kouji (250 µg/kg), sauces (73 µg/kg) and canned coffee (65 µg/kg).

5.1.6 Republic of Korea

The Republic of Korea submitted the results of furan occurrence in foods from a recent publication (Kim et al., 2009b). Furan concentrations were obtained from 407 individual food samples purchased from the local market. Analyses were performed using the GC-MS technique. The limits of the method were 0.4 and 1.4 µg/kg for detection and quantification, respectively. The highest average levels of contamination were found in ground roasted coffee (814.1 µg/kg), canned oyster (181.6 µg/kg), instant coffee (90.1 µg/kg), Korean seasoned pork (63.3 µg/kg) and canned fish (66.7 µg/kg).

5.1.7 Switzerland

Switzerland submitted the results of furan occurrence in foods for the current meeting of the Committee (Kantonales Laboratorium Basel, 2004; Reinhard et al., 2004; SFOPH, 2004). Furan concentrations were obtained from 418 individual food samples purchased in local retail stores. Analyses were performed using the GC-MS technique. The LOQs ranged from 1 to 10 µg/kg. The highest average levels of contamination were found in roasted coffee (powder) (1979 µg/kg), roasted flour/starch (1932.8 µg/kg), instant coffee (powder) (783.3 µg/kg) and caramel (312 µg/kg).

5.1.8 United States of America

The USA submitted the results of furan occurrence in foods from the 2004–2008 Exploratory Data on Furan in Food: Individual Food Products (USFDA, 2009).

Furan concentrations were obtained from 771 individual food samples. Analyses were performed using the GC-MS technique. The minimum and maximum values reported for LOD and LOQ ranged from 0.2 to 3.2 µg/kg and from 0.6 to 9.6 µg/kg, respectively. The highest average levels of contamination were found in baked beans (60.4 µg/kg), soya sauce (52.1 µg/kg), gravies (47.8 µg/kg) and brewed roasted coffee (46.7 µg/kg).

5.1.9 Summary of national occurrence data

The range of national mean levels of furan for foods with the highest contamination levels were as follows: roasted coffee (powder) (814–4590 µg/kg), instant coffee (powder) (90–783 µg/kg), brewed roasted coffee (34–113 µg/kg), baby food (19–96 µg/kg), soya sauce (16–52 µg/kg), canned fish (6–76 µg/kg) and baked beans (27–581 µg/kg). Detailed data are presented in Appendix 1.

6. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

Although the presence of furan as a flavouring component in food was reported in 1979 (Maga, 1979), the first national dietary exposure assessment for furan was not undertaken until 2004, when data on furan concentrations in a variety of foods in the USA became available (USFDA, 2009).

At the present meeting, the Committee considered dietary exposure estimates submitted by the USA (DiNovi & Mihalov, 2007; USFDA, 2009), the EU (EFSA, 2009; Fromberg, Fagt & Granby, 2009) and Brazil (Arisetto, Vicente & Toledo, 2009).

6.1 Furan concentrations used in dietary exposure estimates

Occurrence data are discussed more fully in section 5; however, details relevant to the dietary exposure estimates for furans are discussed below.

For the USA, furan levels were measured in foods prepared as for consumption, and only these foods were included in the dietary exposure assessments for the population in the USA (DiNovi & Mihalov, 2007; USFDA, 2009).

For the European estimates for 14 countries, mean furan levels for broad food groups were derived from the 2004–2009 results for individual foods, and these were then grouped according to the wider food groups described in the EFSA Concise European Food Consumption Database (EFSA, 2008) for use in the dietary exposure assessment (EFSA, 2009). The majority of analyses were for foods as purchased and not necessarily in the form as consumed; for example, most coffee was analysed as instant powder, beans or ground coffee, so a weighted mean furan level for coffee was derived and a dilution factor of 18 applied to convert all coffee as analysed to coffee as drunk. This furan level was then assigned to the wider food group, “coffee, tea and cocoa”.

The level of furan in coffee is of particular interest, as it tends to be higher than levels in most other foods, and therefore the method of deriving the value selected for use in dietary exposure assessments to assign to different types of

coffee can have an impact on estimated dietary exposures. An average level of 42–52 µg/kg was used in the USA estimates for brewed coffee, including decaffeinated coffee, and a higher value of 80 µg/kg was used in the EFSA dietary exposure assessment for all coffee, tea and cocoa (EFSA, 2009).

Different coffees may have different amounts of furans to start with. For example, EFSA (2009) and Crews (2009) reported that green coffee beans contain only traces of furans, whereas high levels of furans are found in full roasted beans (average 3400 µg/kg); grinding coffee beans reduces furan levels by between 10% and 60% (average 1114 µg/kg), and the production of instant coffee powder further reduces furan levels (average 589 µg/kg). These reported levels from European countries were consistent with those reported by Kuballa (2007), Hasnip, Crews & Castle (2006) and Zoller, Sager & Reinhard (2007). The high furan levels in roasted coffee are likely due to the very high temperatures used, which exceed those used in other food processing procedures (Crews & Castle, 2007a). The proportion of furan lost in the making of the coffee from beans varies according to the level of roasting, methods of brewing and equipment used. The furan content of coffee made with closed automatic machines was reported to be higher, at 57–115 µg/kg, than with other systems of brewing: home machine, 9–33 µg/kg; manual machine, 17–24 µg/kg; and French press cafetière, 33–66 µg/kg (Crews & Castle, 2007a; Crews, 2009). The Crews (2009) report also indicates that furan can be inhaled from coffee preparation or cooking and that furan is exhaled at elevated levels after coffee drinking, but there were insufficient data for reliable estimates of intake from air samples.

Recent information from Australia for coffee as drunk supports other values reported in the literature. Furan levels were the lowest for instant coffee (made up), at 2–3 µg/l, and highest for short black coffee, at 210 µg/l, whereas furan levels for other brewed coffees, such as lattes, flat whites and cappuccinos, were reported to be between these values, ranging from 7 to 77 µg/l (FSANZ, 2009). These values were also consistent with the USFDA mean furan values reported for coffee of 1–7 µg/kg for instant coffee (made up) and 34–84 µg/kg for brewed coffee (Morehouse et al., 2008).

Furan levels used in reported dietary exposure assessments for adults in 14 European countries and the USA are given in Table 14.

Table 14. Mean furan content of foods used in dietary exposure assessments

Food group	Europe[a]		USA[b]	
	N	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)
Cereal & cereal products[c]	99	10 LB 14 UB		
Bread			7	0.2
Macaroni & cheese			2	15.9

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Vegetable soups[c]	198	23 LB 24 UB		
Chicken broth			8	11.6
Vegetable beef soup			6	88.0
Vegetables, nuts, pulses, other[c]	344	9 LB 14 UB		
Vegetables[d]	81 14	11.1 (2007–2009) 20.0 (2004–2006)		
Baked beans[d]	18 24	24.1 (2007–2009) 28.3 (2004–2006)	11	84.2
Corn			2 2	1.3 (kernel[e]) 23.0 (creamed)
Green beans[e]			4	5.2
Peas, sweet[e]			3	12.4
Peanut butter			4	6.5
Fruits[c]	84	2 LB 7 UB		
Pears			4	4.9
Pineapples			4	1.9
Fruit cocktail			5	5.9
Peaches			6	5.1
Apple sauce			7	1.9
Fruit & vegetable juices[c]	248	4 LB 7 UB		
Fruit juice[d]	137 66	2.9 (2007–2009) 13.6 (2004–2006)		
Vegetable juice				
Apple juice			7	1.7
Grape juice			6	1.8
Cranberry juice			4	1.3
Tomato juice			4	4.6
Vegetable juice			3	6.3

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Coffee, tea, cocoa as liquid[c]	398	82 LB 82 UB		
Coffee instant (made up)[f]			4 4	1.5 4.5 (decaffeinated)
Coffee brewed[f]			3 3	51.7 41.8 (decaffeinated)
Coffee instant, powder	33 15	437 (2007–2009) 925 (2004–2006)		
Coffee roast bean	9	2272		
Coffee roast ground	66	1114		
Coffee ns	152 123	2384 (2007–2009) 836 (2004–2006)		
Beer & substitutes[c]	86	4 LB 6 UB		
Beer[d]	19 67	8.3 (2007–2009) 4.0 (2004–2006)	8	1.0
Meat & meat products[c]	65	19 LB 22 UB		
Luncheon meat			3	0.9
Canned meat products[e]			5	17.4
Fish & seafood[c]	9	8 LB 10 UB		
Tuna in water[e]			6	5.0
Milk- & dairy-based drinks[c]	20	13 LB 15 UB		
Evaporated milk			3	12.5
Dairy-based products[c]	20	13 LB 15 UB		
Miscellaneous[c]	337	22 LB 23 UB		
Sauces[d]	94 113	7.8 (2007–2009) 16.0 (2004–2006)		
Hot dog chili sauce			2	40.5
Pasta sauce			9	8.7

Table 14 (contd)

Food group	Europe[a]		USA[b]	
	N	Mean concentration (μg/kg)	*N*	Mean concentration (μg/kg)
Pasta & sauce products			7	39.4
Soya sauce[d]	49	25.0 (2007–2009) 29.6 (2004–2006)	6	51.1
Caramel topping			2	3.2
Nutritional diet drinks			20	29.1
Pudding snacks			6	6.4
Gel snacks			3	3.1
Jellies, jams, preserves			30	4.4
Fruit butter spreads			8	18.6
Syrup			7	19.7

LB, lower bound; ns, not specified; UB, upper bound

[a] EFSA (2009). HS-GC-MS and HS-SPME/GC-MS methods of analysis. Years data were collected are given in parentheses.

[b] Morehouse et al. (2008). Furan found in prepackaged, processed adult foods, concentration of 0 μg/kg assigned to non-detect values, HS-GC-MS method of analysis.

[c] Mean furan content according to broad food categories in the EFSA Concise European Food Consumption Database (upper-bound values, non-detect values assigned the LOQ), standard dilution factor of 18 applied to convert coffee beans, grounds, powder to coffee as drunk.

[d] Reported in EFSA (2009). Records used to derive the mean furan levels for broad food groups.

[e] Solid portion, without liquid added by manufacturer.

[f] After preparation, following label instructions.

As the data indicated that heat-processed foods tend to have higher furan levels than do foods prepared by other processes, concern has been expressed about potentially high levels in foods that may be the sole or major source of nutrition for young children, such as infant formula and jarred or canned infant foods (EFSA, 2004, 2009; Morehouse et al., 2008; Arisseto, Vicente & Toledo, 2010). Reported levels of furans in foods sold for young children are summarized in Table 15.

Table 15. Mean furan content of infant foods used in dietary exposure assessments

Food group	Europe[a]		USA[b]		Brazil[c]	
	N	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)	*N*	Mean concentration (µg/kg)
Baby food	447 538	24.2 (2007–2009) 26.4 (2004–2006)			31	1.7–31.8
Apple juice			5	4.0		
Apple sauce			9	4.3		
Sweet potatoes			18	80.3		
Carrots			7	37.6		
Green beans			6	48.2		
Squash			6	49.3		
Garden vegetables			7	75.5		
Dinners with chicken			7	32.9		
Pears			4	5.3		
Bananas			5	21.2		
Infant formula	28 7	21.2 (2007–2009) 9.5 (2004–2006)	4 11 15	0.9 (made up) 7.6 (concentrate) 7.2 (ready to eat)		

[a] EFSA (2009). HS-GC-MS and HS-SPME/GC-MS methods of analysis. Years data were collected are given in parentheses.
[b] Morehouse et al. (2008). Furan found in infant foods, concentration of 0 µg/kg assigned to non-detect values, HS-GC-MS method of analysis.
[c] Arisseto, Vicente & Toledo (2010).

6.2 Estimates of dietary exposure

6.2.1 International estimates of dietary exposure

As furan occurs primarily in heat-processed foods, the Committee noted that international estimates using the GEMS/Food consumption cluster diets could not be generated, as appropriate food consumption data were not available for heat-processed foods across all diets.

6.2.2 National estimates of dietary exposure

Estimates of potential dietary exposure to furans were first reported for Europe in 2004, using the USFDA mean furan concentration data and other available data sources for specific food groups combined with mean food consumption data for the European population as a whole taken from the GEMS/Food diet for Europe (EFSA, 2004; Heppner & Schlatter, 2007; Crews & Castle, 2007a).

Predicted dietary exposure to furan for individual foods from these estimates ranged from 0.04 to 1.93 µg/kg bw per day for coffee, from 0.018 to 0.0378 µg/kg bw per day for vegetables (canned or jarred), from 0.015 to 0.142 µg/kg bw per day for meat products, from 0.007 to 0.009 µg/kg bw per day for bread, from 0.021 to 0.057 µg/kg bw per day for beer, from 0.002 to 0.007 µg/kg bw per day for fruit juices, from 0.003 to 0.007 µg/kg bw per day for fish, from 0.003 to 0.005 µg/kg bw per day for milk and from 0.000 05 to 0.0002 µg/kg bw per day for honey, assuming an average body weight of 60 kg (EFSA, 2004; Crews & Castle, 2007a; Heppner & Schlatter, 2007).

More recent comprehensive reports of dietary exposure at a national level for adults were submitted by the EU to the present meeting for 14 European countries (EFSA, 2009). All these assessments were based on individual dietary records from national nutrition surveys and data collected from 2004 to 2009. Other countries were excluded, as food consumption data were not available for all the relevant food categories. For results expressed per kilogram body weight, individual body weights were used to adjust individual dietary exposure estimates before population statistics were derived. The additional estimate reported for Denmark used the Danish national nutrition survey data combined with new furan data on a number of heat-processed foods from a recent study (Fromberg, Fagt & Granby, 2009), with EFSA data used for other foods (EFSA, 2009).

The dietary exposure estimates submitted by the USA were based on 2004 and 2007 furan data. In the first estimate, the furan concentrations reported in 2004 for foods as consumed were combined with relevant food consumption amounts from 2-day individual records of 24 h recall from the 1994–1998 Continuing Survey of Food Intakes by Individuals (CSFII) surveys (Morehouse et al., 2008). These were updated in the second estimates using more recent furan data collected in 2007 (DiNovi & Mihalov, 2007).

Dietary exposure estimates for furan for adults in 14 European countries and for the whole population and children in Denmark and the USA are summarized in Table 16.

For adults, the 2009 estimates of mean dietary exposure to furan for 14 European countries ranged from 0.29 to 1.17 µg/kg bw per day. Ninety-fifth-percentile dietary exposures to furan ranged from 0.60 to 2.22 µg/kg bw per day (EFSA, 2009).

A more accurate dietary exposure estimate for Danish adults indicated mean dietary exposures to furan for adults of 27 µg/day (median 33.5 µg/day) or 0.39 µg/kg bw per day (median 0.48 µg/kg bw per day), assuming a 70 kg body weight for adults (Fromberg, Fagt & Granby, 2009). In this study, the 95th-percentile dietary exposure to furan was estimated for mean and high furan levels for coffee

Table 16. Estimates of dietary exposure to furan

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
Europe (summary)[a]	Concise European Food Consumption Database (adults)	0.29 min LB 0.72 median LB 1.17 max LB	0.60 min LB 1.68 median LB 2.22 max LB	Broad food categories (foods assigned analytical values to represent all foods in category, weighted means used for some food groups) Individual dietary and body weight records
	Furan levels collated from 2004–2009 records	0.29 min UB 0.72 median UB 1.17 max UB	0.69 min UB 1.75 median UB 2.27 max UB	
Austria	EFSA (2009)	0.73 LB 0.80 UB	1.77 LB 1.83 UB	24 h recall, 2005–2006, adults
Belgium	EFSA (2009)	0.67 LB 0.72 UB	1.56 LB 1.69 UB	2 × 24 h recall, 2004–2005, adults
Bulgaria	EFSA (2009)	0.29 LB 0.34 UB	0.65 LB 0.73 UB	24 h recall, 2004, adults
Czech Republic	EFSA (2009)	0.79 LB 0.85 UB	1.54 LB 1.60 UB	2 × 24 h recall, 2003–2004, adults
Denmark	EFSA (2009)	0.95 LB 1.02 UB	2.10 LB 2.19 UB	EFSA (2009): adults Fromberg, Fagt & Granby (2009): 7 day, pre-coded diary with open fields, 2000–2004, 70 kg bw for adults, assumed 20 kg bw for children aged 4–6 years
	Fromberg, Fagt & Granby (2009)	0.39 (0.48 median) adults	0.97–1.82	
	Fromberg, Fagt & Granby (2009)	0.08 (0.06 median) children 4–6 years		

Table 16 (contd)

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
France	EFSA (2009)	0.57 LB 0.63 UB	1.23 LB 1.30 UB	7-day dietary record, 2006–2007, adults
Great Britain	EFSA (2009)	0.98 LB 1.04 UB	2.00 LB 2.05 UB	7-day dietary record, 2000–2001, adults
Hungary	EFSA (2009)	0.38 LB 0.44 UB	0.86 LB 0.92 UB	3-day dietary record, 2003–2004, adults
Iceland	EFSA (2009)	0.63 LB 0.68 UB	1.61 LB 1.67 UB	Adults
Ireland	EFSA (2009)	0.99 LB 1.06 UB	1.85 LB 1.93 UB	7-day dietary record, 1997–1999, adults
Italy	EFSA (2009)	0.32 LB 0.39 UB	0.60 LB 0.69 UB	3-day dietary record, 2005–2006, adults
Netherlands	EFSA (2009)	1.17 LB 1.23 UB	2.22 LB 2.27 UB	2 × 24 h recall, 2003, adults
Poland	EFSA (2009)	1.01 LB 1.08 UB	1.85 LB 1.93 UB	24 h recall, 2000, adults
Slovakia	EFSA (2009)	0.70 LB 0.75 UB	1.75 LB 1.81 UB	24 h recall, 2006, adults
USA[b]	CSFII 1994–1998, whole population over 2 years, two 24 h recalls (>5000 subjects) 2004 USFDA furan levels (USFDA, 2009)	0.25 mean 2+ years 0.23 mean 2–5 years 0.24 mean 15–45 years		Individual dietary records and body weights, only foods with analytical values assigned a furan level Analytical values for foods as consumed

Table 16 (contd)

Country/region	Data source	Mean exposure (μg/kg bw per day)	95th-percentile exposure (μg/kg bw per day)	Comments
USA[c]	CSFII 1994–1998, whole population over 2 years, two 24 h recalls (>5000 subjects) 2007 USFDA furan levels (DiNovi & Mihalov, 2007)	0.26 mean 2+ years 0.41 mean 0–1 years	0.61 90th percentile 2+ years 0.99 90th percentile 0–1 years	Individual dietary records and body weights, only foods with analytical values assigned a furan level Analytical values for foods as consumed

LB, lower bound; max, maximum; min, minimum; UB, upper bound

[a] EFSA (2009). LB values assume that a concentration of 0 μg/kg is assigned to non-detects, whereas UB values assume that the LOQ is assigned to non-detects.

[b] Morehouse et al. (2008).

[c] DiNovi & Mihalov (2007).

and ranged from 0.97 µg/kg bw per day (mean furan level for coffee of 38 µg/kg) to 1.82 µg/kg bw per day (high furan level for coffee of 72 µg/kg), assuming a 70 kg body weight for adults. For Danish children aged 4–6 years, estimated mean dietary exposures were 1.5 µg/day (median 1.1 µg/day); body weights were not reported for this age group. If an average 20 kg body weight is assumed, then estimated mean dietary exposure would be 0.08 µg/kg bw per day (median 0.06 µg/kg bw per day).

Mean dietary exposure to furan for the whole population in the USA from the 2004 study was 0.25 µg/kg bw per day, and for adults 15–45 years of age, 0.24 µg/kg bw per day. The estimated mean dietary exposure to furan for children aged 2–5 years from this study was also very similar, at 0.23 µg/kg bw per day (Morehouse et al., 2008). The updated 2007 USA estimate of mean dietary exposure of 0.26 µg/kg bw per day for the whole population was consistent with the earlier findings (DiNovi & Mihalov, 2007). Additional results were also presented in the second study, with a 90th-percentile dietary exposure estimate of 0.61 µg/kg bw per day for the whole population; for infants aged 0–1 years, estimated mean dietary exposure was 0.41 µg/kg bw per day, and the 90th-percentile exposure was 0.99 µg/kg bw per day.

Estimates of dietary exposure to furan for infants in Europe and Brazil assumed that all food consumed by the infants other than infant formula had been in jars or cans; results are summarized in Table 17. These estimates were considered appropriate for infants fed solely on these products, but would be overestimates of dietary exposure for the whole infant population.

Predicted dietary exposures for European infants and children aged 3–12 months were modelled and reported by EFSA (2004, 2009), with 95th-percentile consumption results for 2009 given for two different scenarios for infants aged 6 and 9 months.

In 2004, predicted furan dietary exposures for a 6-month-old child, assuming consumption of 273 g jarred food (Kersting et al., 1998) and 870 g infant formula (EC, 2003), ranged from <0.03 to 3.5 µg/kg bw per day, using minimum and maximum furan levels reported by the USFDA in 2004 (USFDA, 2009).

In 2009, EFSA predicted dietary furan exposures for infants aged 3–12 months based on food consumption amounts for infant formula and jarred foods reported by Kersting et al. (1998) and furan levels for European countries collated from 2004–2009 records. Mean dietary exposures were 0.27 µg/kg bw per day for a 3-month-old infant, 0.93 µg/kg bw per day for a 6-month-old infant, 1.01 µg/kg bw per day for a 9-month-old infant and 0.69 µg/kg bw per day for a 12-month-old infant. Ninety-fifth-percentile dietary exposures to furan were predicted for 6- and 9-month-old infants by assuming either 95th-percentile consumption of infant formula and mean consumption of jarred foods or vice versa. For 6-month-old infants, predicted 95th-percentile exposures ranged from 1.14 to 1.34 µg/kg bw per day, and for 9-month-old infants, from 1.15 to 1.26 µg/kg bw per day; in both cases, dietary exposure was higher in the scenario where jarred foods were consumed at the 95th-percentile amount. Estimated dietary furan exposures for infants aged 6–11 months in Brazil were similar to those reported for European countries, ranging

Table 17. Predicted upper-bound dietary exposure to furans for infants

Country/region	Data source	Mean exposure (µg/kg bw per day)	95th-percentile exposure (µg/kg bw per day)[a]	Comments
Europe[b]	Kersting et al. (1998), consumption of infant formula and jarred food Furan levels collated from 2004–2009 records	0.27 3 months 0.93 6 months 1.01 9 months 0.69 12 months	— 1.14 6 months scenario 1 1.34 6 months scenario 2 1.15 9 months scenario 3 1.26 9 months scenario 4 —	Higher body weights for boys were used[c]
Europe[d]	Kersting et al. (1998), consumption of jarred food, infant formula amount from EC (2003) 2004 USFDA furan levels (USFDA, 2009)	<0.03 minimum to 3.5 maximum 6 months		Range reflects maximum and minimum furan levels reported Body weight of 7.5 kg for 6-month-old child
Brazil (Arisseto, Vicente & Toledo, 2010)	Analysed jarred baby food, recommended and actual food consumption	0.46–0.82	1.34–2.40 (99th percentile)	Range based on actual food consumption (lower estimate) and recommended consumption of jarred food (upper estimate) Body weight of 8.4 kg for 6- to 11-month-old child

[a] Scenarios 1, 3: sum of 95th-percentile consumption of infant formula and mean consumption of jarred foods for each age group; scenarios 2, 4: sum of mean consumption of infant formula and 95th-percentile consumption of jarred foods for each age group. Note factor of 0.119 used to convert dry infant formula reported by Kersting et al. (1998) to formula made up.

[b] EFSA (2009).

[c] Body weights for boys assumed: 6.4 kg, 3-month-old infant; 8.1 kg, 6-month-old infant; 9.3 kg, 9-month-old infant; 10.3 kg, 12-month-old infant.

[d] EFSA (2004); Heppner & Schlatter (2007).

from 0.42 to 0.82 µg/kg bw per day for mean exposure and from 1.34 to 2.40 µg/kg bw per day at the 99th percentile. The lower estimate was based on actual food consumption amounts, and the upper estimate on recommended food consumption amounts for infants in Brazil (Arisseto, Vicente & Toledo, 2010).

6.3 Contributors to dietary furan exposure

For adults, coffee was the major contributor to dietary furan exposure, likely to be between 40% and 80% of total dietary exposure (Crews & Castle, 2007a; Heppner & Schlatter, 2007; Morehouse et al., 2008; EFSA, 2009; Fromberg, Fagt & Granby, 2009). In the EFSA (2009) estimates, other contributors to dietary furan exposure (>5%) were reported to be cereal and cereal products (3–16%), vegetables, nuts, pulses and others (2–14%), meat and meat products (4–13%) and milk and milk-based drinks (2–9%).

For Danish children aged 4–6 years, the major contributors to dietary furan exposure were breakfast cereals (40%), sauces (14%), cake (13%), stew (11%) and fruit juice (10%). If the higher furan levels recently reported by Fromberg, Fagt & Granby (2009) are used for toasted bread instead of the mean furan level for bread, this food group would make a higher contribution.

For infants from 6 months of age, baby food sold in jars or cans was the most important contributor to dietary furan exposure (Arisseto, Vicente & Toledo, 2010).

7 DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

7.1 Identification of key data for risk assessment

7.1.1 Pivotal data from biochemical and toxicological studies

(a) Metabolism and activation

About 80% of radiolabelled furan administered by intragastric instillation to rats was rapidly eliminated in the first 24 h, with expired air, urine and faeces all being significant routes of excretion. The great majority of the administered dose remaining in tissues (19%) was found in the liver (13%), with much smaller amounts also found in kidney, blood and small and large intestines, each accounting for less than 1% of the initial dose. In plasma and liver, no unchanged furan was present, and 80% was not extractable with organic solvents, suggesting that radioactive label from furan was bound to macromolecules.

CYP2E1 was identified as the major route of oxidation of furan. Hepatocytes from mice oxidized furan at a greater rate than those from rats or humans. The history of the human donors suggested that the variability between individuals in the rate of oxidation of furan was due to ethanol induction of CYP2E1 in two of the three individuals. Such variability might affect the extent of first-pass uptake and bioactivation following oral exposure.

Production of carbon dioxide was postulated to occur through ring opening of furan by oxidation to BDA, with subsequent formation of maleic acid followed by metabolism to carbon dioxide. The rapid metabolism of furan to carbon dioxide suggested that some of the non-extractable radioactivity in tissues could be due to amino acids in proteins derived from citric acid intermediates. The formation of the oxidative metabolite, BDA, which is extremely reactive, was confirmed in rat liver microsomes both in vitro and in vivo, and BDA was found to be rapidly conjugated with GSH. Characterization of urinary metabolites of furan from the rat suggested that in addition to its reaction with GSH, BDA readily reacts with cysteine and lysine residues in protein. The characterization of the metabolites provided evidence for alkylation of proteins by BDA through internal protein cysteine–BDA–lysine crosslinking and GSH–BDA–protein crosslinking. Other evidence for crosslinking (protein–protein, protein–DNA or DNA–DNA) is available. This, of course, would be expected for BDA, an unsaturated dialdehyde. In addition, nucleoside adducts of BDA with dCyd and dAdo have been characterized from model systems and then identified in DNA digests treated with physiologically relevant concentrations of BDA, as well as in DNA isolated from *Salmonella typhimurium* strain TA104 that had been treated with mutagenic concentrations of BDA used in the Ames assay in a dose-related manner. The nucleoside adducts of dAdo were of particular concern because of its formation of substituted etheno adducts, in the process exposing a reactive aldehyde moiety. Such etheno adducts have been implicated in the induction of point mutations and the generation of DNA–DNA and DNA–protein crosslinks.

As an early, critical, cytotoxic effect in hepatocytes, furan was found to uncouple oxidative phosphorylation in mitochondria both in vitro and in vivo.

(b) Toxicity

Single-dose and short-term repeated-dose studies clearly identify the liver as the target organ of furan toxicity by the oral route in the mouse and rat. The rat was notably more sensitive than the mouse to the toxicity of furan in terms of time to onset of liver changes and severity and incidence of lesions at a particular dose. Typically, the lesions in the rat affected the caudate and left lateral lobes of the liver and were characterized by hyperplasia and cholangiofibrosis of the biliary tract, greenish-yellow to brown pigmentation of Kupffer cells and cytomegaly, degeneration and necrosis of hepatocytes. These lesions occurred at doses that were less than those associated with increased liver weights. Nodular hyperplasia was also observed in the rat. Although furan induced the same types of hepatocelluar lesions in both species, the most prominent lesions in the rat affected the biliary tract, whereas in mice, hepatocytes and the biliary tract were equally affected by furan administration. Also, at the higher doses in the rat, renal tubular lesions were noted, as well as atrophy of the thymus, testes and ovaries; none of these effects were noted in the mouse.

A 90-day (i.e. 13-week) study in F344 rats (Gill et al., 2009) extended the dose range from that used in the 13-week NTP (1993) assays (i.e. 2–8 mg/kg bw) to as low as 0.03 mg/kg bw per day, 5 days/week. Mild subcapsular hepatic lesions consisting of rare to occasional apoptosis of hepatocytes, Kupffer cells filled with

yellow pigment and microfoci of inflammatory cells were noted at the next highest dose of 0.12 mg/kg bw per day, 5 days/week. In addition, statistically significant dose-related changes in clinical chemistry parameters related to liver function, altered thymocyte maturation and dose-related increases in platelet count (both sexes) and dose-related increases of serum thyroxine, testosterone and LH levels and of intratesticular testosterone levels (males only) were noted. Although statistically increased intratesticular testosterone levels were found at all doses tested, no adverse effects on testosterone synthesis were noted. Thus, the lowest dose tested (0.03 mg/kg bw per day, 5 days/week) appears to be a no-observed-adverse-effect level (NOAEL). Preliminary results suggested that furan affects the mRNA expression levels of StAR protein, which regulates cholesterol transfer within the mitochondria, the rate-limiting step of steroid hormone production. This is relevant to the finding that uncoupling of oxidative phosphorylation in hepatocyte mitochondria is an early, critical event in the cytolethality of furan.

In long-term studies, male rats displayed reductions in red blood cell parameters at 9 months and bone marrow hyperplasia, whereas mice were less affected.

(c) Mutagenicity and clastogenicity

Furan has been extensively tested for genotoxicity, with mixed results. In in vitro tests, results were mainly negative for DNA alteration, bacterial mutagenicity and mammalian mutagenicity. The few positive results (e.g chromosomal alteration) were accompanied by negative results for the same end-point. In vivo, furan did not induce DNA alteration, mutagenicity in *Drosophila* or bone marrow chromosomal aberrations. Mixed results were reported for SCEs in mouse bone marrow but not rat bone marrow, and increased micronuclei were reported in mouse splenocytes, but not mouse bone marrow. BDA was positive in vitro for DNA alteration, bacterial gene mutation and mammalian cell gene mutation, but not mammalian cell micronucleus induction.

(d) Carcinogenicity

Furan was carcinogenic in mice and rats, inducing tumours of the liver in both species. In mice administered furan intragastrically for 2 years, hepatocellular adenomas and carcinomas were induced by 8 mg/kg bw per day (44/50) and 15 mg/kg bw per day (50/50) in males, compared with 26/50 in controls. In females, the incidences were 34/50 and 50/50, respectively, compared with 7/50 in controls. In rats dosed intragastrically for 2 years, furan induced primarily cholangiocarcinomas: in males, 43/50 at 2 mg/kg bw per day, 48/50 at 4 mg/kg bw per day and 49/50 at 8 mg/kg bw per day, compared with 0/50 in controls; and in females, 49/50 at 2 mg/kg bw per day, 50/50 at 4 mg/kg bw per day and 48/50 at 8 mg/kg bw per day, compared with 0/50 in controls. Hepatocellular adenomas and carcinomas were present in males at incidences of 1/50 controls, 5/50 low dose, 22/50 mid dose and 35/50 high dose. In females, the incidences were 0/50, 2/50, 4/50 and 8/50, respectively. In addition, mononuclear cell leukaemias were increased in both sexes.

The induction of cholangiocarcinomas in rats, which did not occur in mice, appears to be related to the earlier development of cholangiofibrosis only in rats.

(e) Special studies

Furan did not bind to DNA in the rat liver, although it bound to liver microsomal protein in vitro. In vitro, BDA reacted with amino acids, GSH and 2′-deoxynucleosides. In studies of DNA crosslinking, negative results were obtained for furan in vivo and mixed results for BDA in vitro.

7.2 BMD analysis of tumour dose–response data

Dosing experimental animals with furan in bioassays gave rise to increases in liver tumours and leukaemias. The neoplasms evaluated were as follows: cholangiocarcinomas in livers of male and female rats, hepatocellular neoplasms in male and female rats, mononuclear cell leukaemias in male and female rats and hepatocellular neoplasms in male and female mice.

The cholangiocarcinomas were seen only in rats and were associated with extreme hepatotoxicity and an early and marked biliary tract response. The relevance for humans of the cholangiocarcinomas is not clear, and the available data do not allow for an analysis of mode of action. Also, the high incidences of these neoplasms at all doses of furan precluded identification of a point of departure. The Committee was aware of ongoing studies in rats to extend the dose–response data and address mechanistic aspects.

The modelled data are shown in Table 18. In the dose–response analysis, the United States Environmental Protection Agency (USEPA) benchmark dose (BMD) software (BMDS version 2.0) was used. Benchmark doses for 10% extra risk (BMD_{10}) of tumours and their 95% lower confidence limits ($BMDL_{10}$) were calculated by fitting the nine different statistical models to the experimental data considered relevant. The slope parameter for the log-logistic and the log-probit models was restricted to be ≥1. Only BMD_{10} and $BMDL_{10}$ were considered further, which resulted from models with an acceptable fit to the data, based on statistical considerations (chi-squared test, $P > 0.1$). The BMD_{10} and $BMDL_{10}$ (in milligrams per kilogram of body weight per day) as well as the goodness of fit criteria resulting from fitting the models to the data are tabulated, and a graph resulting from the model giving the lowest BMDL is shown.

Table 19 shows the modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the study of Moser et al. (2009). All models except the quantal-linear model gave an acceptable fit to the data, as indicated by the high *P*-value of the chi-squared test. The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold, and a graphical representation of the model fit is shown for the multistage model, the model resulting in the lowest $BMDL_{10}$ (Figure 2). The BMD_{10} and $BMDL_{10}$ ranged from 1.87 to 2.86 mg/kg bw per day and from 1.34 to 1.89 mg/kg bw per day, respectively.

Table 18. Experimental data used for BMD modelling

Dose (mg/kg bw per day)[a]	Incidence	Sex	Species	Reference
Hepatocellular adenoma and carcinoma				
0	1/50	Male	Rat	NTP (1993)
2	5/50	Male	Rat	NTP (1993)
4	22/50	Male	Rat	NTP (1993)
8	35/50	Male	Rat	NTP (1993)
0	0/50	Female	Rat	NTP (1993)
2	2/50	Female	Rat	NTP (1993)
4	4/50	Female	Rat	NTP (1993)
8	8/50	Female	Rat	NTP (1993)
0	26/50	Male	Mouse	NTP (1993)
8	44/50	Male	Mouse	NTP (1993)
15	50/50	Male	Mouse	NTP (1993)
0	7/50	Female	Mouse	NTP (1993)
8	34/50	Female	Mouse	NTP (1993)
15	50/50	Female	Mouse	NTP (1993)
0	3/36	Female	Mouse	Moser et al. (2009)
0.5	8/72	Female	Mouse	Moser et al. (2009)
1	6/53	Female	Mouse	Moser et al. (2009)
2	5/41	Female	Mouse	Moser et al. (2009)
4	12/36	Female	Mouse	Moser et al. (2009)
8	29/39	Female	Mouse	Moser et al. (2009)
Leukaemias				
0	8/50	Male	Rat	NTP (1993)
2	11/50	Male	Rat	NTP (1993)
4	17/50	Male	Rat	NTP (1993)
8	25/50	Male	Rat	NTP (1993)
0	8/50	Female	Rat	NTP (1993)
2	9/50	Female	Rat	NTP (1993)
4	17/50	Female	Rat	NTP (1993)
8	21/50	Female	Rat	NTP (1993)

Table 18 (contd)

Dose (mg/kg bw per day)[a]	Incidence	Sex	Species	Reference
Cholangiocarcinoma				
0	0/50	Male	Rat	NTP (1993)
2	43/50	Male	Rat	NTP (1993)
4	48/50	Male	Rat	NTP (1993)
8	49/50	Male	Rat	NTP (1993)
0	0/50	Female	Rat	NTP (1993)
2	49/50	Female	Rat	NTP (1993)
4	50/50	Female	Rat	NTP (1993)
8	48/50	Female	Rat	NTP (1993)

[a] Dosing was 5 days/week.

Table 19. Modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the Moser et al. (2009) study

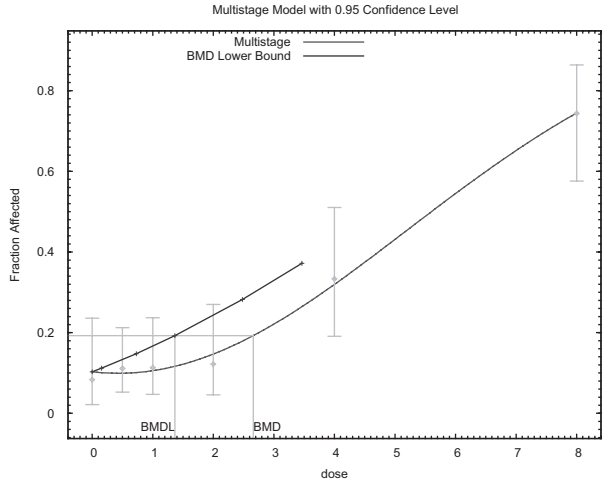
	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	235.33	233.88	235.29	235.23	235.50	233.64	234.19	235.47	241.56
Chi-square	0.36	0.88	0.33	0.27	0.53	0.66	1.17	0.50	8.01
P-value	0.95	0.93	0.96	0.97	0.91	0.96	0.88	0.92	0.09
BMD_{10}[a]	2.76	2.03	2.78	**2.86**	2.66	2.34	**1.87**	2.62	0.96
$BMDL_{10}$[a]	1.65	1.71	1.77	**1.89**	**1.34**	1.34	1.59	1.53	0.74

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

The modelling outputs for hepatocellular adenoma and carcinoma for male and female mice treated with furan in the NTP (1993) study are shown in Table 20 and Table 21, respectively. For male mice, six out of the nine models gave an acceptable fit, and the quantal-linear model resulted in the lowest $BMDL_{10}$ (Figure 3). The BMD_{10} and $BMDL_{10}$ ranged from 0.49 to 6.66 mg/kg bw per day and from 0.35 to 1.85 mg/kg bw per day, respectively. For female mice, five out of the nine models gave an acceptable fit, and the multistage model resulted in the lowest $BMDL_{10}$ (Figure 4). The BMD_{10} and $BMDL_{10}$ ranged from 1.63 to 6.88 mg/kg bw per day and from 1.07 to 4.20 mg/kg bw per day, respectively.

Figure 2. Multistage model for hepatocellular adenoma and carcinoma in female mice treated with furan in the Moser et al. (2009) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Table 20. Modelling output for hepatocellular adenoma and carcinoma for male mice treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	109.93	111.78	109.93	111.93	110.24	NC	111.02	111.93	112.50
Chi-square	0.00	1.17	0.00	0.00	0.18	NC	0.70	0.00	1.69
P-value	0.97	0.28	0.99	NA	0.67	NC	0.40	NA	0.19
BMD_{10}[a]	4.97	0.71	**6.66**	6.20	2.14	NC	0.78	4.13	**0.49**
$BMDL_{10}$[a]	0.45	0.53	**1.85**	1.43	0.42	NC	0.61	0.45	**0.35**

AIC, Akaike's information criterion; NA, not assessed; NC, not calculated, as the number of observations is lower than the number of parameters in the model

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

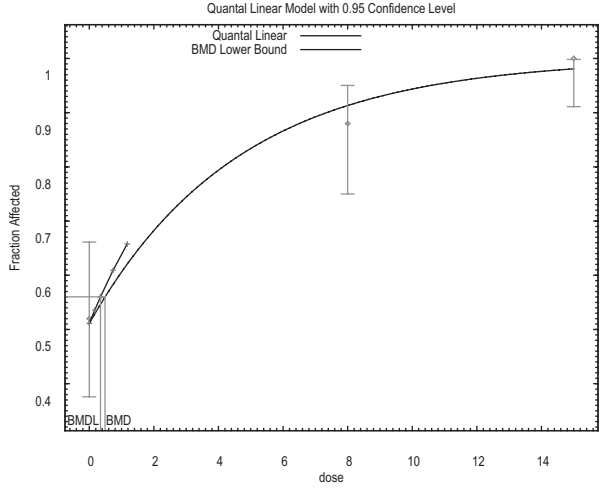
Table 21. Modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	107.22	110.65	107.18	109.18	109.23	NA	109.45	109.18	116.72
Chi-square	0.02	2.19	0.00	0.00	1.26	NA	1.49	0.00	6.56
P-value	0.90	0.14	0.99	NA	0.26	NA	0.22	NA	0.01
BMD_{10}[a]	5.37	1.77	**6.88**	6.60	2.44	NA	**1.63**	4.85	0.61
$BMDL_{10}$[a]	2.99	1.35	**4.20**	3.79	**1.07**	NA	1.29	2.17	0.48

AIC, Akaike's information criterion; NA, not assessed; NC, not calculated, as the number of observations is lower than number of parameters in the model

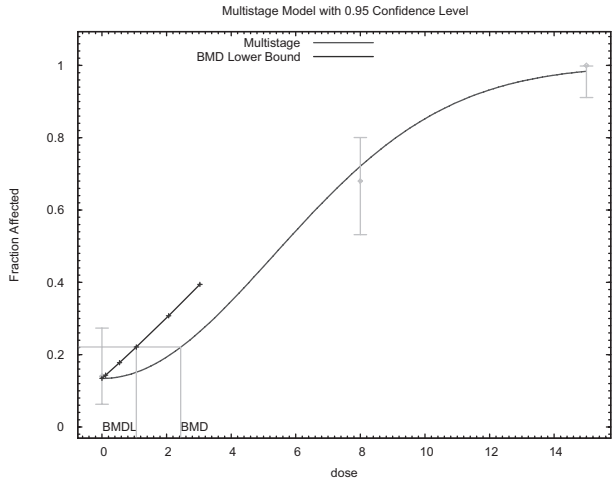
[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 3. Quantal-linear model for hepatocellular adenoma and carcinoma in male mice treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Figure 4. Multistage model for hepatocellular adenoma and carcinoma in female mice treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

The modelling outputs for hepatocellular adenoma and carcinoma for male and female rats treated with furan in the NTP (1993) study are shown in Table 22 and Table 23, respectively. For male rats, the model fits were generally poor, and only four out of the nine models just passed the goodness of fit test criterion of $P > 0.1$ and thus gave an acceptable fit. The Weibull model resulted in the lowest $BMDL_{10}$ (Figure 5), and the BMD_{10} and $BMDL_{10}$ ranged from 1.64 to 1.92 mg/kg bw per day and from 1.00 to 1.34 mg/kg bw per day, respectively. In female rats, all models gave an acceptable fit. The BMD_{10} for female rats tended to be higher than the BMD_{10} for the other sex–species combinations. The BMD_{10} and $BMDL_{10}$ ranged from 4.82 to 6.47 mg/kg bw per day and from 3.16 to 5.25 mg/kg bw per day, respectively. The log-logistic model resulted in the lowest $BMDL_{10}$ (Figure 6).

The modelling outputs for the mononuclear cell leukaemias for male and female rats treated with furan in the NTP (1993) study are given in Table 24 and Table 25, respectively. The mononuclear leukaemias in rats, which are notable in the strain used in the NTP (1993) bioassay, are of unknown pathogenesis, and the increases occurred against a background of unusually low incidences in the control groups. Moreover, studies of genotoxicity in rat bone marrow, where the progenitor cells of the leukaemias presumably arise, were negative, and there is no plausible mode of action. All models gave an acceptable fit to the data. For male rats, the BMD_{10} and $BMDL_{10}$ ranged from 1.66 to 2.47 mg/kg bw per day and from 0.97 to 1.98 mg/kg bw per day, respectively. For female rats, the respective figures are

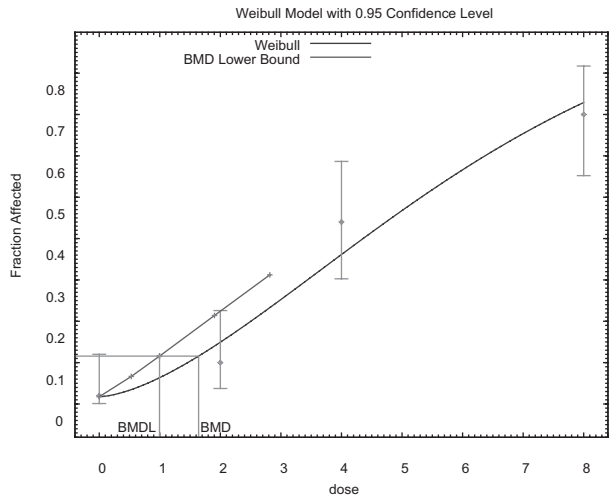
Table 22. Modelling output for hepatocellular adenoma and carcinoma for male rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	180.07	183.40	179.41	179.11	181.16	181.16	182.14	180.58	183.15
Chi-square	2.06	7.25	1.40	1.11	3.11	3.11	6.18	2.53	6.16
P-value	0.15	0.03	0.24	0.29	0.08	0.08	0.05	0.11	0.05
BMD_{10}[a]	1.78	2.19	1.85	**1.92**	1.57	1.57	2.06	**1.64**	0.84
$BMDL_{10}$[a]	1.08	1.81	1.23	**1.34**	0.87	0.87	1.71	**1.00**	0.68

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 5. Weibull model for hepatocellular adenoma and carcinoma in male rats treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

2.13–2.98 mg/kg bw per day and 1.18–2.29 mg/kg bw per day, respectively. For male and female rats, the Weibull and the log-logistic models resulted in the lowest $BMDL_{10}$, respectively (Figures 7 and 8).

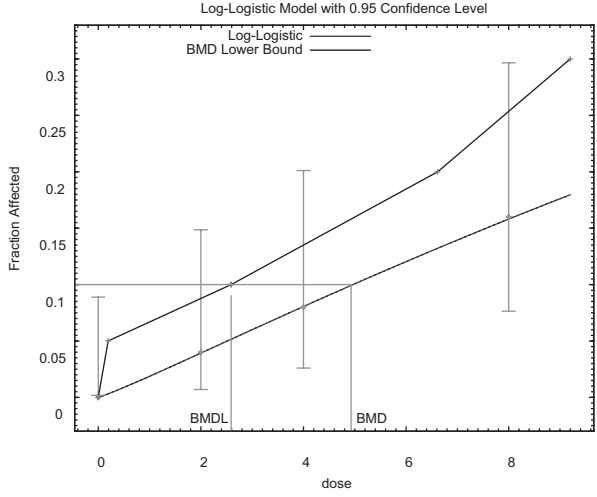
Table 23. Modelling output for hepatocellular adenoma and carcinoma for female rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	92.64	94.98	92.64	92.65	94.64	94.64	94.65	92.64	90.65
Chi-square	0.00	1.53	0.00	0.01	0.00	0.00	1.33	0.00	0.01
P-value	1.00	0.47	1.00	1.00	1.00	1.00	0.52	1.00	1.00
BMD_{10}	4.96	**6.47**	4.93	**4.82**	5.00	5.00	6.23	4.96	4.94
$BMDL_{10}$	3.28	**5.25**	**3.16**	3.93	3.28	3.28	4.97	3.28	3.28

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 6. Log-logistic model for hepatocellular adenoma and carcinoma in female rats treated with furan in NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

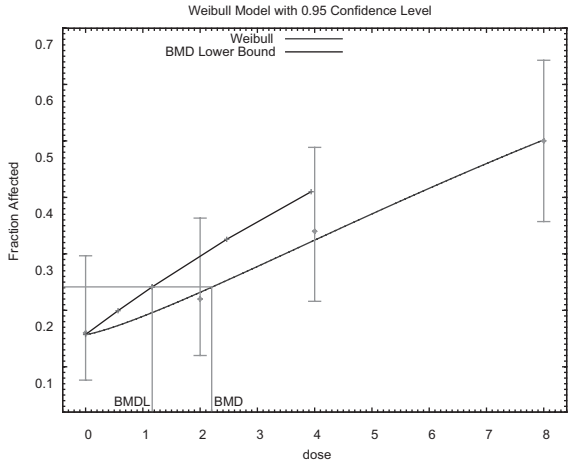
Table 24. Modelling output for leukaemias for male rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	236.16	234.32	236.12	234.24	236.22	236.22	234.27	236.17	234.41
Chi-square	0.09	0.24	0.06	0.16	0.14	0.14	0.20	0.10	0.32
P-value	0.77	0.89	0.81	0.92	0.71	0.71	0.91	0.76	0.85
BMD_{10}[a]	2.24	**2.47**	2.26	2.32	2.11	2.11	2.35	2.20	**1.66**
$BMDL_{10}$[a]	1.16	1.98	**0.97**	**1.98**	1.16	1.16	1.88	1.16	1.15

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 7. Weibull model for leukaemias in male rats treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

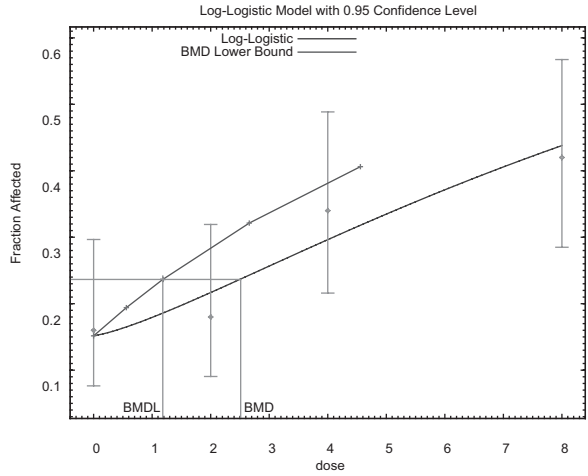
Table 25. Modelling output for leukaemias for female rats treated with furan in the NTP (1993) study

	Gamma	Logistic	Log-logistic	Log-probit	Multistage	Multistage cancer	Probit	Weibull	Quantal-linear
AIC	230.26	228.56	230.19	228.41	230.34	230.34	228.49	230.28	228.36
Chi-square	1.02	1.33	0.95	1.20	1.09	1.09	1.27	1.03	1.09
P-value	0.31	0.51	0.33	0.55	0.30	0.30	0.53	0.31	0.58
BMD_{10}[a]	2.54	**2.98**	2.51	2.58	2.27	2.27	2.86	2.49	**2.13**
$BMDL_{10}$[a]	1.40	**2.29**	**1.18**	2.32	1.39	1.39	2.18	1.40	1.39

AIC, Akaike's information criterion

[a] The BMD_{10} and $BMDL_{10}$ giving the lowest and highest accepted values are indicated in bold.

Figure 8. Log-logistic model for leukaemias in female rats treated with furan in the NTP (1993) study



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Table 26 summarizes the modelling output for all end-points considered and lists the ranges of BMD_{10}s and $BMDL_{10}$s. The BMD_{10}s and $BMDL_{10}$s derived from the different data were broadly similar. Those for the hepatocellular adenomas and carcinomas in male mice were the lowest but varied over a broad range, and there was a high incidence of liver tumours in the control male mice. The study of Moser

Table 26. Ranges of BMD_{10} and $BMDL_{10}$ values for tumours associated with administration of furan by gavage

Tumour type	Study	Sex and species	BMD_{10}[a] (mg/kg bw per day)	$BMDL_{10}$[a] (mg/kg bw per day)
Hepatocellular adenomas and carcinoma	Moser et al. (2009)	Female mice	1.87–2.86	1.34–1.89
	NTP (1993)	Male mice	0.49–6.66	0.35–1.85
	NTP (1993)	Female mice	1.63–6.88	1.07–4.20
	NTP (1993)	Male rat	1.64–1.92	1.00–1.34
	NTP (1993)	Female rat	4.82–6.47	3.16–5.25
Leukaemia	NTP (1993)	Male rat	1.66–2.47	0.97–1.98
	NTP (1993)	Female rat	2.13–2.98	1.18–2.29

BMD_{10}, benchmark dose for 10% extra risk of tumours; $BMDL_{10}$, 95% lower confidence limit for the benchmark dose for 10% extra risk of tumours. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

[a] BMD_{10}s and $BMDL_{10}$s have not been adjusted for the dosing schedule of 5 days/week.

et al. (2009) had more and lower doses and a greater number of animals in the low-dose group compared with the NTP (1993) studies. A comparison of the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) and NTP (1993) studies with the dosages used in each study indicates that those derived from the Moser et al. (2009) study are much closer to the dosage levels than those in the NTP (1993) study. This indicates that the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) study had less uncertainty than those derived from the NTP (1993) studies. Therefore, the Committee decided to use the $BMDL_{10}$ of 1.3 mg/kg bw per day, derived from the hepatocellular adenoma/carcinoma data from the Moser et al. (2009) study, as the point of departure.

8. COMMENTS

8.1 Absorption, distribution, metabolism and excretion

Following oral administration to mice and rats, furan is rapidly absorbed, metabolized and eliminated in urine and faeces as metabolites and exhaled in air as unchanged furan and carbon dioxide formed as a result of ring opening. The initial ring-opened metabolite is BDA, which is formed in the liver in a reaction catalysed by CYP2E1. Furan-derived products are most abundant in the liver of dosed animals. A variety of identified urinary metabolites could arise from amino acid or protein crosslinking.

8.2 Toxicological data

The toxicity of orally administered furan has been extensively studied in mice and rats over a wide dose range. The primary site of toxicity of furan is the liver, although the kidneys and lungs are also affected at high doses (>30 mg/kg bw per day). In addition, changes in some haematological and hormonal parameters occur at doses as low as 0.12 mg/kg bw per day administered 5 days/week.

Regarding hepatotoxicity, uncoupling of hepatocyte mitochrondrial oxidative phosphorylation is an early critical event in cytolethality. Liver cell injury, including oxidative stress, progresses to cell death. This, in turn, gives rise to regenerative responses, including increased hepatocellular proliferation in mice and rats and, notably in the rat, an early proliferative reaction involving the biliary epithelium, referred to as cholangiofibrosis. These proliferative changes may be the basis for liver tumorigenicity, either alone or in combination with DNA alteration. Although furan is not genotoxic in a number of test systems and binding to rat liver DNA was not detectable, the metabolite BDA is highly reactive and binds to proteins and nucleic acids. BDA produced DNA strand breaks in cultured mammalian cells and was mutagenic in bacteria and cultured mammalian cells; being a dialdehyde, it also formed crosslinks with DNA of cultured cells. The in vitro genotoxicity of BDA allows the possibility that BDA formed in vivo from furan could react with DNA.

Several cancer bioassays of orally (gavage) administered furan in mice and rats have been performed. In mice, doses of 8 and 15 mg/kg bw per day, administered 5 days/week (NTP, 1993), and 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, administered 5 days/week (Moser et al., 2009), were used. In rats, doses of 2, 4 and 8 mg/kg bw per day were administered 5 days/week (NTP, 1993). In livers of male and female rats, high incidences of cholangiocarcinomas were induced at all doses in the NTP (1993) study, accompanied by biliary tract hyperplasia, metaplasia and fibrosis. Hepatocellular neoplasms were increased at lower incidences. In both sexes of rat, furan also increased the incidences of mononuclear cell leukaemia, albeit against unusually low background incidences in control groups. In male and female mice in both studies, only hepatocellular neoplasms were increased.

8.3 Observations in humans

No epidemiological studies were available.

8.4 Analytical methods

GC-MS has been shown to be the most suitable technique for the reliable detection of low levels of furan in foods. GC-MS is usually preceded by HS extraction or HS-SPME. Both HS and HS-SPME approaches are simple and convenient and give satisfactory results for analyses of volatiles. Owing to the high volatility of furan, food samples and standards need to be chilled and handled quickly. Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples have to be homogenized. Most published methods include the use of deuterium-labelled furan as an internal standard, which is normally added to the homogenized sample before the extraction. LODs and LOQs from 0.1 to 5 ng/g and from 0.4 to 13 ng/g, respectively, have been reported for methods based

on HS extraction. Lower LODs and LOQs are reported for methods using HS-SPME. No certified reference material is currently available.

8.5 Formation, effects of processing and fate in foods

Furan can be formed in a variety of foods from different precursors by thermal and non-thermal processing (ionizing radiation). The proposed routes for furan formation are mainly based on 1) Maillard reactions, 2) thermal degradation of carbohydrates, 3) thermal degradation of certain amino acids, 4) thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids and 5) free radical reactions during irradiation. Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure-cooking conditions (sterilization, 121 °C, 25 min), and pH plays a complex role in the mechanism of furan formation. For coffee, the amount of furan formed in beans varies according to the level of roasting. Grinding may reduce furan levels by 10–60%, and further decreases occur in the production of instant coffee powder and in brewing.

Limited data are available on the formation of furan in home-cooked food as well as on the stability of furan during cooking, storing and reheating of meals. As furan appears to be well dissolved within the matrix, opening the jars (e.g. baby foods) exposes only a relatively small surface area. Therefore, despite furan's volatility, its evaporation is hindered by its slow diffusion inside the food matrix. However, if canned or jarred foods are heated in a saucepan under stirring, larger declines of furan content can be observed. Studies on the losses of furan during warming procedures for ready-to-eat foods have shown conflicting results, with some authors reporting losses of 29–85% and others finding that furan persists during normal heating practices. Losses of furan in heated foods left for cooling seem to be insignificant.

8.6 Levels and patterns of contamination in food commodities

Furan concentration data covering 21 countries were submitted by Australia, Brazil, Canada, the EU, Japan, the Republic of Korea, Switzerland and the USA. The total number of analytical results (single or composite samples) evaluated at the present meeting was 5662, with 59.8% from Europe, 16.7% from North America, 22.8% from Asia, 1.0% from Latin America and 0.7% from the Pacific region. The occurrence of furan has been investigated mainly in thermally processed foods, such as coffee, canned and jarred foods, including baby foods, soups and sauces. The ranges of national mean levels of furan for the foods with the highest contamination levels were as follows: roasted coffee (powder), 814–4590 µg/kg; instant coffee (powder), 90– 783 µg/kg; brewed roasted coffee, 34–113 µg/kg; jarred baby foods, 19–96 µg/kg; soya sauce, 16–52 µg/kg; canned fish, 6–76 µg/kg; and baked beans, 27–581 µg/kg. Lower levels have been found in other foods, including products from vegetables, meat, milk and cereals.

8.7 Food consumption and dietary exposure assessment

Although the presence of furan as a flavour component in food was first reported in 1979, dietary exposure assessments for furan were not undertaken until 2004, when data on furan concentrations in a variety of foods in the USA became available.

At the present meeting, the Committee considered dietary exposure estimates for furan submitted by the USA, the EU and Brazil, all of which were based on analysed data for foods and individual dietary records for the populations of interest. The dietary exposure estimates for the whole population, infants and young children in the USA and Denmark were considered by the Committee to underestimate dietary exposure, as furan levels were assigned to the specific foods analysed only and hence did not represent the whole food supply. In contrast, the dietary exposure estimates for adults submitted by the EU for 14 European countries were considered to be overestimates; as the mean furan levels from 2004–2009 results for individual foods were grouped and then assigned to the food consumption amount for the relevant wider food group, as described in the EFSA Concise European Food Consumption Database, some uncertainty was introduced in these dietary exposure assessments. For example, the furan level for coffee was assigned to the wider food group "coffee, tea and cocoa"; as levels of furan are much higher in coffee than in either tea or cocoa, this results in an overestimate of dietary exposure to furan from these beverages.

For infants and young children, concern has been expressed about potential dietary exposure to furan from the consumption of baby foods sold in jars or cans. Estimates of dietary exposure to furan for infants in Europe and Brazil assumed that all food consumed by the infants had been in jars or cans; these estimates were considered appropriate for infants fed solely on these products, but would be overestimates of dietary exposure for the whole infant population.

As furan occurs primarily in heat-processed foods, the Committee noted that international estimates using the GEMS/Food consumption cluster diets could not be generated, as appropriate food consumption data were not available for heat-processed foods.

In general, mean dietary exposure to furan from national assessments ranged from 0.25 to 1.17 µg/kg bw per day for adults, from 0.08 to 0.23 µg/kg bw per day for children 1–6 years of age and from 0.27–1.01 µg/kg bw per day for infants up to 12 months of age. For consumers at high percentiles of dietary exposure, estimates ranged from 0.60 to 2.22 µg/kg bw per day for adults and from 0.99 to 1.34 µg/kg bw per day for infants; no high-percentile dietary exposure data were available for children. Estimates of dietary exposure to furan are summarized in Table 27.

For adults, coffee was the major contributor to dietary furan exposures (40–80%), with cereals, vegetables, meats and dairy foods contributing more than 5% to total exposure. For children, breakfast cereals were the major contributor (40%). As reported furan levels were much higher for brewed coffee than for ready-to-drink instant coffee, the type of coffee consumed in a given population and the

Table 27. Estimates of dietary exposure to furan

Country	Dietary exposure estimate (µg/kg bw per day)	
	Mean	Upper percentile
Europe		
Europe[a]	0.29–1.17 adults 0.27–1.01 infants 3–12 months	0.60–2.22 adults (95th) 1.14–1.34 infants 6–9 months (95th)
Denmark[b]	0.95–1.02 adults 0.08 children 4–6 years	2.10–2.19 adults (95th)
North America		
USA[c]	0.25–0.26 adults 0.23 children 2–5 years 0.41 infants 0–12 months	0.61 adults (90th) 0.99 infants 0–12 months (90th)
South America		
Brazil[d]	0.46 infants 6–11 months	1.34 infants 6–11 months (99th)

[a] Individual dietary records for 14 European countries from the Concise European Food Consumption Database; analysed furan values from period 2004–2009.
[b] Individual dietary records from the Danish National Nutrition Survey; new furan data for some heat-processed foods; EFSA data for other foods.
[c] Individual dietary records from the USA 1994–1996, 1998 supplementary CSFII; analysed furan values from 2003 and 2007 surveys.
[d] Individual dietary records for infants; analysed data for baby food.

furan level for coffee influenced the dietary exposure estimates for adults. The lower values obtained in the USFDA estimates for adults compared with those from EFSA for European countries were largely explained by the lower furan level for brewed coffee in the USFDA estimates. Despite this, estimated dietary furan exposures available to the Committee were in the same order of magnitude.

For the purposes of risk characterization, a value of 1 µg/kg bw per day was taken to represent mean dietary exposure to furan, and a value of 2 µg/kg bw per day was taken to represent high dietary exposure. The Committee considered these values to be sufficient to cover potential dietary exposures of infants and children to furan.

8.8 Dose–response analysis

Dosing with furan in bioassays gave rise to increases in liver tumours and leukaemias. The neoplasms evaluated for dose–response analysis were as follows: cholangiocarcinomas in livers of male and female rats, hepatocellular neoplasms in male and female rats, mononuclear cell leukaemias in male and female rats and hepatocellular neoplasms in male and female mice.

The cholangiocarcinomas were seen only in rats and were associated with extreme hepatotoxicity and an early and marked biliary tract proliferative response.

The relevance for humans of the cholangiocarcinomas is not clear, and the available data do not allow for an analysis of the mode of action. Also, the high incidences of these neoplasms at all doses of furan precluded identification of a point of departure. The Committee was aware of ongoing studies in rats to extend the dose–response data and address mechanistic aspects for this end-point.

The mononuclear cell leukaemias in rats, which occur in high incidence in the strain used in the NTP (1993) bioassay, are of unknown pathogenesis, and the increases occurred against a background of unusually low incidences in the control groups. Moreover, studies of genotoxicity in rat bone marrow, where the progenitor cells of the leukaemias presumably arise, were negative, and the mode of action is unknown.

The hepatocellular neoplasms in rats in the NTP (1993) study and in mice in the NTP (1993) study and the study of Moser et al. (2009) and the leukaemias in rats in the NTP (1993) study were selected for modelling.

In the dose–response analysis using the USEPA BMD software (BMDS version 2.0), the nine different statistical models were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on statistical considerations (chi-squared test, $P > 0.1$) were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure resulted in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (see Table 26 above).

The BMD_{10}s and $BMDL_{10}$s derived from the different data were broadly similar. Those for the hepatocellular adenomas and carcinomas in male mice were the lowest but varied over a broad range, and there was a high incidence of liver tumours in the control male mice. The study of Moser et al. (2009) had more and lower doses and a greater number of animals in the low-dose group compared with the NTP (1993) studies. For each study, a comparison of the BMD_{10}s and $BMDL_{10}$s derived with the dosages used indicates that those derived from the Moser et al. (2009) study are much closer to the dosage levels used in that study. This indicates that the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. (2009) study had less uncertainty than those derived from the NTP (1993) studies. Therefore, the Committee decided to use the $BMDL_{10}$ of 1.34 mg/kg bw per day, which corresponds to 0.96 mg/kg bw per day when adjusted from a 5 days/week dosing schedule to an average daily dose, in female mice derived from the hepatocellular adenoma and carcinoma data from the Moser et al. (2009) study as the point of departure.

9. EVALUATION

Margins of exposure (MOEs) were calculated at dietary exposures of 0.001 mg/kg bw per day, to represent the average dietary exposure to furan for the general population, and 0.002 mg/kg bw per day, to represent the dietary exposure to furan for consumers with high exposure. This estimate will also cover dietary exposure of children. Comparison of these dietary exposures with the $BMDL_{10}$ of 0.96 mg/kg bw per day for induction of hepatocellular adenomas and carcinomas

in female mice gives MOEs of 960 and 480 for average and high dietary exposures, respectively. The Committee considered that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

The furan levels can be reduced in some foods through volatilization (e.g. by heating and stirring canned/jarred foods in an open saucepan). However, there is currently a lack of quantitative data for all foods, and no information is available on other mitigation methods.

10. REFERENCES

Altaki MS, Santos FJ, Galceran MT (2007). Analysis of furan in foods by headspace solid-phase microextraction–gas chromatography–ion trap mass spectrometry. *Journal of Chromatography A*, 1146:103–109.

Altaki MS, Santos FJ, Galceran MT (2009). Automated headspace solid-phase microextraction versus headspace for the analysis of furan in foods by gas chromatography–mass spectrometry. *Talanta*, 78:1315–1320.

Arisseto AP, Vicente E, Toledo MCF (2009). Furan in selected foods in Brazil. Data submitted to FAO/WHO by Brazilian Health Surveillance Agency/Ministry of Health (ANVISA).

Arisseto AP, Vicente E, Toledo MCF (2010). Determination of furan levels in commercial samples of baby food from Brazil and preliminary risk assessment. *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(8): 1051–1059.

Becalski A, Seaman S (2005). Furan precursors in food: a model study and development of a simple headspace method for determination of furan. *Journal of AOAC International*, 88:102–106.

Becalski A et al. (2005). Development and validation of a headspace method for determination of furan in food. *Food Additives and Contaminants*, 22:535–540.

Becalski A et al. (2009). Development of analytical method and survey of foods for furan, 2-methylfuran and 3-methylfuran with estimated exposure. Data submitted to FAO/WHO by Health Canada, Ottawa, Ontario.

Bianchi F et al. (2006). Development and validation of a solid phase micro-extraction–gas chromatography–mass spectrometry method for the determination of furan in baby-food. *Journal of Chromatography A*, 1102:268–272.

Bononi M, Tateo F (2009). Determination of furan by headspace solid-phase microextraction–gas chromatography–mass spectrometry in balsamic vinegars of Modena (Italy). *Journal of Food Composition and Analysis*, 22:79–82.

Borlak J, Thum T (2001). Induction of nuclear transcription factors, cytochrome P450 monooxygenases, and glutathione *S*-transferase alpha gene expression in Aroclor 1254–treated rat hepatocyte cultures. *Biochemical Pharmacology*, 61(2):145–153.

Burka LT, Washburn KD, Irwin RD (1991). Disposition of [^{14}C]furan in the male F344 rat. *Journal of Toxicology and Environmental Health*, 34(2):245–257.

Byrns MC, Predecki DP, Peterson LA (2002). Characterization of nucleoside adducts of *cis*-2-butene-1,4-dial, a reactive metabolite of furan. *Chemical Research in Toxicology*, 15:373–379.

Byrns MC, Vu CC, Peterson LA (2004). The formation of substituted 1,N6-etheno-2′-deoxyadenosine and 1,N2-etheno-2′-deoxyguanosine adducts by *cis*-2-butene-1,4-dial, a reactive metabolite of furan. *Chemical Research in Toxicology*, 17:1607–1613.

Byrns MC et al. (2006). Detection of DNA adducts derived from the reactive metabolite of furan, *cis*-2-butene-1,4-dial. *Chemical Research in Toxicology*, 19:414–420.

Carfagna MA, Held SD, Kedderis GL (1993). Furan-induced cytolethality in isolated rat hepatocytes: correspondence with in vivo dosimetry. *Toxicology and Applied Pharmacology*, 123(2):265–273.

Chen LJ, Hecht SS, Peterson LA (1995). Identification of *cis*-2-butene-1,4-dial as a microsomal metabolite of furan. *Chemical Research in Toxicology*, 8(7):903–906.

Chen LJ, Hecht SS, Peterson LA (1997). Characterization of amino acid and glutathione adducts of *cis*-2-butene-1,4-dial, a reactive metabolite of furan. *Chemical Research in Toxicology*, 10(8):866–874.

Chromtech (2006). *SPDE: the magic needle*. Idstein, Chromtech Analytical Instruments (http://www.labicom.cz/Upload/1736.pdf).

Cooke GM (2009). Effects of furan on male rat reproduction parameters: 90 day gavage study. In: *Investigations on toxicity of furan—a preliminary report*. Ottawa, Ontario, Health Canada, Food Directorate, pp. 66–78.

Crews C (2009). *Consumer exposure to furan from heat-processed foods and kitchen air*. Scientific/technical report submitted to the European Food Safety Authority by the Food and Environment Research Agency, United Kingdom (EFSA-Q-2009-00847; http://www.efsa.europa.eu/en/scdocs/doc/30e.pdf).

Crews C, Castle L (2007a). A review of the occurrence, formation and analysis of furan in heat processed food. *Trends in Food Science and Technology*, 18:365–372.

Crews C, Castle L (2007b). The determination of furan in foods—challenges and solutions. *LCGC Europe*, 20:498.

Crews C et al. (2007). Factors affecting the analysis of furan in heated foods. *Food Additives and Contaminants*, 24:108–113.

Curran IHA et al. (2009). Effects of oral furan administration on liver gene and protein expression in Fischer 344 rats. In: *Investigations on toxicity of furan—a preliminary report*. Ottawa, Ontario, Health Canada, Food Directorate, pp. 42–55.

DiNovi M, Mihalov MS (2007). *An updated exposure assessment for furan from consumption of adult and baby foods*. Silver Spring, MD, United States Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Furan/ucm110770.htm).

Durling LJK, Svensson K, Abramsson-Zetterberg L (2007). Furan is not genotoxic in the micronucleus assay in vivo or in vitro. *Toxicology Letters*, 169(1):43–50.

EC (2003). *Report of the Scientific Committee on Food (SCF) on the revision of essential requirements of infant formulae and follow-on formulae (adopted on 4 April 2003).* Brussels, European Commission (SCF/CS/NUT/IF/65 Final).

EFSA (2004). Report of the scientific panel on contaminants in the food chain on provisional findings on furan in food. Parma, European Food Safety Authority. *EFSA Journal*, 137:1–20.

EFSA (2008). *Concise European Food Consumption Database*. Parma, European Food Safety Authority (http://www.efsa.europa.eu/en/datex/datexfooddb.htm).

EFSA (2009). *Results of the monitoring of furan levels in food, a report of the Data Collection and Exposure Unit in response to a request from the European Commission*. Parma, European Food Safety Authority, pp. 1–23 (EFSA Scientific Report 304; http://www.efsa.europa.eu/en/scdocs/doc/304r.pdf).

Eicher T, Hauptmann S, Speicher A (2003). Five-membered heterocycles: furan. In: *The chemistry of heterocyclics—structures, reactions, synthesis and applications*, 1st ed. Weinheim, Wiley-VCH, pp. 53–63.

Elmore LW, Sirica AE (1991). Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Research*, 51:5752–5759.

Elmore LW, Sirica AE (1993). “Intestinal type” of adenocarcinoma preferentially induced in right/caudate liver lobes of rats treated with furan. *Cancer Research*, 53(2):254–259.

Escobar-Garcia D et al. (2001). S9 induction by the combined treatment with cyclohexanol and albendazole. *Mutagenesis*, 16(6):523–528.

Fan X (2005a). Impact of ionizing radiation and thermal treatments on furan levels in fruit juices. *Journal of Food Science*, 70:E409–E414.

Fan X (2005b). Formation of furan from carbohydrates and ascorbic acid following exposure to ionizing radiation and thermal processing. *Journal of Agricultural and Food Chemistry*, 53:7826–7831.

Fan X, Geveke DJ (2007). Furan formation in sugar solution and apple cider upon ultraviolet treatment. *Journal of Agricultural and Food Chemistry*, 55:7816–7821.

Fan X, Mastovska K (2006). Effectiveness of ionizing radiation in reducing furan and acrylamide levels in foods. *Journal of Agricultural and Food Chemistry*, 54:8266–8270.

Fan X, Sokorai KJ (2008). Effect of ionizing radiation on furan formation in fresh-cut fruits and vegetables. *Journal of Food Science*, 73:C79–C83.

Fan X, Sommers CH (2006). Effect of gamma radiation on furan formation in ready-to-eat products and their ingredients. *Journal of Food Science*, 71:C407–C412.

Fan X, Huang L, Sokorai KJB (2008). Factors affecting thermally induced furan formation. *Journal of Agricultural and Food Chemistry*, 56:9490–9494.

FAO/WHO (2008). *Report of the 2nd Session of the Codex Committee on Contaminants in Food. The Hague, The Netherlands, 31 March – 4 April 2008*. Rome, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 08/31/41; http://www.codexalimentarius.net/web/archives.jsp?year=08).

Foureman P et al. (1994). Chemical mutagenesis testing in *Drosophila.* IX. Results of 50 coded compounds tested for the National Toxicology Program. *Environmental and Molecular Mutagenesis*, 23(1):51–63.

Fransson-Steen R et al. (1997). Furan-induced cell proliferation and apoptosis in B6C3F1 mouse liver. *Toxicology*, 118:195–204.

Fromberg A, Fagt S, Granby K (2009). *Furan in heat processed food products including home cooked food products and ready-to-eat products.* Scientific report submitted to the European Food Safety Authority by the Danish National Food Institute (EFSA-Q-2009-00846; http://www.efsa.europa.eu/en/scdocs/doc/1e.pdf).

FSANZ (2009). Furan content of coffee. Data submitted to FAO/WHO by Food Standards Australia New Zealand.

Gammal LM et al. (1984). Toxicity–distribution relationships among 3-alkylfurans in the mouse lung. *Toxicology*, 30:177–184.

Gill S et al. (2009). Subchronic oral toxicity "90-day study" to determine effects of furan in Fischer 344 rats. In: *Investigations on toxicity of furan—a preliminary report*. Ottawa, Ontario, Health Canada, Food Directorate, pp. 5–41.

Gingipalli L, Dedon PC (2001). Reaction of *cis*- and *trans*-2-butene-1,4-dial with 2′-deoxycytidine to form stable oxadiazabicyclooctaimine adducts. *Journal of the American Chemistry Society*, 123:2664–2665.

Goldmann P et al. (2005). Rapid determination of furan in heated foodstuffs by isotope dilution solid phase micro-extraction–gas chromatography–mass spectrometry (SPME-GC-MS). *Analyst*, 130:878–883.

Halila S et al. (2005). Fine-tuning furan toxicity: fast and quantitative DNA interchain cross-link formation upon selective oxidation of a furan containing oligonucleotide. *Chemical Communications (Cambridge, England)*, 7:936–938.

Hamadeh HK et al. (2004). Integration of clinical and gene expression endpoints to explore furan-mediated hepatotoxicity. *Mutation Research*, 549:169–183.

Hasnip C, Crews C, Castle L (2006). Some factors affecting the formation of furan in heated foodstuffs. *Food Additives and Contaminants*, 24(Suppl. 1):219–227.

Heppner CW, Schlatter J (2007). Data requirements for risk assessment of furan in food. *Food Additives and Contaminants*, 24(Suppl. 1):114–121.

Hickling KC et al. (2010a). Evidence of oxidative stress and associated DNA damage, increased proliferative drive, and altered gene expression in rat liver produced by the cholangiocarcinogenic agent furan. *Toxicologic Pathology*, 38(2):230–243.

Hickling KC et al. (2010b). Induction and progression of cholangiofibrosis in rat liver injured by oral administration of furan. *Toxicologic Pathology*, 38(2):213–229.

Ho IP, Yoo SJ, Tefera S (2005). Determination of furan levels in coffee using automated solid-phase microextraction and gas chromatography/mass spectrometry. *Journal of AOAC International*, 88:574–576.

Hoenicke K et al. (2004). Analysis of furan in different foodstuffs using gas chromatography mass spectrometry. *Czech Journal of Food Science*, 22(Special issue):357–358.

HSDB (2001). Furan. In: *Hazardous Substances Data Bank.* Bethesda, MD, United States National Library of Medicine (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB).

Jestoi M et al. (2009). Furan in the baby-food samples purchased from the Finnish markets—Determination with SPME-GC-MS. *Food Chemistry*, 117:522–528.

Jin X, Kearns N, Coughlan MC (2009). Effects of furan on markers of systemic oxidative stress and antioxidant defense in rats. In: *Investigations on toxicity of furan—a preliminary report*. Ottawa, Ontario, Health Canada, Food DIrectorate, pp. 56–41.

Johansson E et al. (1997). Frequency of Ha-*ras*-1 gene mutations inversely correlated with furan dose in mouse liver tumors. *Molecular Carcinogenesis*, 18:199–205.

Jun HJ et al. (2008). Correlation of urinary furan with plasma γ-glutamyltranspeptidase levels in healthy men and women. *Food and Chemical Toxicology*, 46:1753–1759.

Kantonales Laboratorium Basel (2004). Furan in food. Data submitted to FAO/WHO.

Kedderis GL (1997). Extrapolation of in vitro enzyme induction data to humans in vivo. *Chemico-Biological Interactions*, 107:109–121.

Kedderis GL, Held SD (1996). Prediction of furan pharmacokinetics from hepatocyte studies: comparison of bioactivation and hepatic dosimetry in rats, mice, and humans. *Toxicology and Applied Pharmacology*, 140:124–130.

Kedderis GL et al. (1993). Kinetic analysis of furan biotransformation by F-344 rats in vivo and in vitro. *Toxicology and Applied Pharmacology*, 123(2):274–282.

Kellert M et al. (2008a). Biomarkers of furan exposure by metabolic profiling of rat urine with liquid chromatography–tandem mass spectrometry and principal component analysis. *Chemical Research in Toxicology*, 21:761–768.

Kellert M et al. (2008b). Tests for genotoxicity and mutagenicity of furan and its metabolite *cis*-2-butene-1,4-dial in L5178Y $tk^{+/-}$ mouse lymphoma cells. *Mutation Research*, 657:127–132.

Kersting M et al. (1998). Measured consumption of commercial infant food products in German infants: results from the DONALD study. *Journal of Pediatric Gastroenterology & Nutrition*, 27(5):547–552.

Kim T-K et al. (2009a). Effect of cooking or handling conditions on the furan levels of processed foods. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26:767–775.

Kim T-K et al. (2009b). Furan in commercially processed foods: four-year field monitoring and risk assessment study in Korea. *Journal of Toxicology and Environmental Health*, 72:1304–1310.

Kuballa T (2007). [Furan in coffee and other foods.] *Journal für Verbraucherschulz und Lebensmittelsicherheit*, 2:429–433 (in German).

Kuballa T, Ruge W (2005). *Untersuchungsmethoden zu Furangehalten in Kaffee.* Deutscher Lebensmittelchemikertag, Hamburg, September, pp. 19–21 [cited in Vranová & Ciesarová, 2009].

Kuballa T, Stefan S, Nina S (2005). Furan concentrations in coffee and coffee beverages. *Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht*, 101:229–235.

Kubiak A, Karasek L, Wenzl T (2008). *Proficiency test on the determination of furan in baby food*. Luxembourg, Office for Official Publications of the European Communities (JRC Scientific and Technical Reports, EUR 2354 EN; http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/11420/1/jrc48419%20%20eur%2023544.pdf).

Lachenmeier DW, Reusch H, Kuballa T (2009). Risk assessment of furan in commercially jarred baby foods, including insights into its occurrence and formation in freshly home-cooked foods for infants and young children. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26:776–785.

La Pera L et al. (2009). Analysis of furan in coffee of different provenance by head-space solid phase microextraction gas chromatography–mass spectrometry: effect of brewing procedures. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26:786–792.

Leopardi P et al. (2010). Assessment of in vivo genotoxicity of the rodent carcinogen furan: evaluation of DNA damage and induction of micronuclei in mouse splenocytes. *Mutagenesis*, 25(1):57–62.

Limacher A et al. (2007). Formation of furan and methylfuran from ascorbic acid in model systems and food. *Food Additives and Contaminants*, 24:122–135.

Limacher A et al. (2008). Formation of furan and methylfuran by Maillard-type reactions in model systems and food. *Journal of Agricultural and Food Chemistry*, 56:3639–3647.

Lu D, Peterson L (2010). Identification of furan metabolites derived from cysteine–*cis*-2-butene-1,4-dial–lysine cross-links. *Chemical Research in Toxicology*, 23:142–151.

Lu D et al. (2009). Degraded protein adducts of *cis*-2-butene-1,4-dial are urinary and hepatocyte metabolites of furan. *Chemical Research in Toxicology*, 22:997–1007.

MAFF (2009a). Occurrence—aggregate data on furan in foods. Data submitted to FAO/WHO by the Ministry of Agriculture, Forestry and Fisheries of Japan.

MAFF (2009b). Analytical results—furan concentration in foods. Data submitted to FAO/WHO by the Ministry of Agriculture, Forestry and Fisheries of Japan.

Maga JA (1979). Furan in foods. *CRC Critical Reviews in Food Science and Nutrition*, 11:355–400.

Marinari UM, Ferro M, Sciaba L (1984). DNA-damaging activity of biotic and xenobiotic aldehydes in Chinese hamster ovary cells. *Cell Biochemistry and Function*, 2(4):243–248.

Märk J et al. (2006). Quantitation of furan and methylfuran formed in different precursor systems by proton transfer reaction mass spectrometry. *Journal of Agricultural and Food Chemistry*, 54:2786–2793.

McGregor DB et al. (1988). Responses of the L5178Y tk+/tk− mouse lymphoma cell forward mutation assay. III. 72 coded chemicals. *Environmental and Molecular Mutagenesis*, 12(1):85–154.

McMurtry RJ, Mitchell JR (1977). Renal and hepatic necrosis after metabolic activation of 2-substituted furans and thiophenes, including furosemide and cephaloridine. *Toxicology and Applied Pharmacology*, 42:285–300.

Morehouse K et al. (2008). Survey of furan in heat processed foods by headspace gas chromatography–mass spectrometry and estimated adult exposure. *Food Additives and Contaminants*, 25(3):259–264.

Mori Y et al. (2001). *N*-Benzylimidazole for preparation of S9 fraction with multi-induction of metabolizing enzymes in short-term genotoxicity assays. *Mutagenesis*, 16(6):479–486.

Mortelmans K et al. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environmental Mutagenesis*, 8(Suppl. 7):1–119.

Moser GJ et al. (2009). Furan-induced dose–response relationships for liver cytotoxicity, cell proliferation, and tumorigenicity (furan-induced liver tumorigenicity). *Experimental and Toxicologic Pathology*, 61(2):101–111.

Mosesso P (2009). *Deliverable 3.2: Determination of the presence of chromosomal aberrations and sister chromatid exchanges (SCE's) in bone marrow cells (WP 3.1).* Project co-funded by the European Commission within the Sixth Framework Programme (http://www.furan-ra.toxi.uni-wuerzburg.de/furan_ra_2007_public_area/deliverablesresults_with_dissemination_level_public/deliverable_32/).

Mugford CA, Carfagna MA, Kedderis GL (1997). Furan-mediated uncoupling of hepatic oxidative phosphorylation in Fischer-344 rats: an early event in cell death. *Toxicology and Applied Pharmacology*, 144(1):1–11.

NTP (1993). *Toxicology and carcinogenesis studies of furan (CAS No. 110-00-9) in F344 rats and B6C3F1 mice (gavage studies).* Research Triangle Park, NC, United States Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Environmental Health Sciences, National Toxicology Program, 286 pp. (Technical Report Series No. 402; NIH Publication No. 93-2857).

Nyman PJ et al. (2006). Single-laboratory validation of a method for the determination of furan in foods by using static headspace sampling and gas chromatography/mass spectrometry. *Journal of AOAC International*, 89:1417–1424.

Nyman PJ et al. (2008). Single-laboratory validation of a method for the determination of furan in foods by using headspace gas chromatography/mass spectrometry, part 2—Low-moisture snack foods. *Journal of AOAC International*, 91:414–421.

Parmar D, Burka LT (1993). Studies on the interaction of furan with hepatic cytochrome P-450. *Journal of Biochemical Toxicology*, 8(1):1–9.

Perez Locas C, Yaylayan VA (2004). Origin and mechanistic pathways of formation of the parent furan—a food toxicant. *Journal of Agricultural and Food Chemistry*, 52:6830–6836.

Peterson LA, Naruko KC, Predecki DP (2000). A reactive metabolite of furan, *cis*-2-butene-1,4-dial, is mutagenic in the Ames assay. *Chemical Research in Toxicology*, 13(7):531–534.

Peterson LA et al. (2005). Glutathione trapping to measure microsomal oxidation of furan to *cis*-2-butene-1,4-dial. *Drug Metabolism and Disposition*, 33(10):1453–1458.

Peterson LA et al. (2006). Identification of a *cis*-2-butene-1,4-dial-derived glutathione conjugate in the urine of furan-treated rats. *Chemical Research in Toxicology*, 19(9): 1138–1141.

Ravindranath V, Burka LT, Boyd MR (1984). Reactive metabolites from the bioactivation of toxic methylfurans. *Science*, 224:884–886.

Reinhard H et al. (2004). Furan in foods on the Swiss market—method and results. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, 95:532–535.

Ridgway K, Lalljie SPD, Smith RM (2006). Comparison of in-tube sorptive extraction techniques for non-polar volatile organic compounds by gas chromatography with mass spectrometric detection. *Journal of Chromatography A*, 1124:181–186.

Roberts D et al. (2008). Effect of consumer cooking on furan in convenience foods. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25:25–31.

Senyuva HZ, Gökmen V (2005). Analysis of furan in foods: is headspace sampling a fit-for-purpose technique? *Food Additives and Contaminants*, 22:1198–1202.

Senyuva HZ, Gökmen V (2007). Potential of furan formation in hazelnuts during heat treatment. *Food Additives and Contaminants*, 24:136–142.

SFOPH (2004). Overview of furan determinations. Data submitted to FAO/WHO by the Swiss Federal Office of Public Health.

Shirey RE (1999). SPME fibers and selection for specific applications. In: Wercinski SAS, ed. *Solid-phase microextraction: a practical guide.* New York, NY, Marcel Dekker, pp. 59–110.

Slack GC, Snow NH, Kou D (2003). Extraction of volatile organic compounds from solids and liquids: In: Mitra S, ed. *Sample preparation techniques in analytical chemistry.* Hoboken, NJ, John Wiley & Sons, pp. 184–200.

Stich HF et al. (1981). Clastogenicity of furans found in food. *Cancer Letters*, 13(2):89–95.

USFDA (2004). *Determination of furan in foods* [method dated 7 May 2004; modified 2 June 2005 and 27 October 2006]. Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Furan/ucm078400.htm).

USFDA (2009). *Exploratory data on furan in food: individual food products*. Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Furan/ucm078439.htm).

Vranová J, Ciesarová Z (2009). Furan in food—a review. *Czech Journal of Food Science*, 27:1–10.

Vranová J, Bednáriková A, Ciesarová Z (2007). In-house validation of a simple headspace gas chromatography–mass spectrometry method for determination of furan levels in food. *Journal of Food and Nutrition Research*, 46:123–127.

Wenzl T (2008). *Methods for the determination of furan in food. Outcome of a survey conducted among EU food control laboratories.* Luxembourg, Office for Official Publications of the European Communities (JRC Scientific and Technical Reports, EUR 23269 EN).

Wenzl T, Lachenmeier DW, Gökmen V (2007). Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. *Analytical and Bioanalytical Chemistry*, 389:119–137.

Wiley RA et al. (1984). Toxicity–distribution relationships among 3-alkylfurans in mouse liver and kidney. *Toxicology and Applied Pharmacology*, 74:1–9.

Wilson DM et al. (1992). Evaluation of genotoxicity, pathological lesions and cell proliferation in livers of rats and mice treated with furan. *Environmental and Molecular Mutagenesis*, 19(3):209–222.

Yaylayan VA (2006). Precursors, formation and determination of furan in food. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 1:5–9.

Yoshida I et al. (2007). Rapid and improved determination of furan in baby foods and infant formulas by headspace GC/MS. *Journal of the Food Hygienic Society of Japan*, 48:83–89.

Zoller O, Sager F, Reinhard H (2007). Furan in food: headspace method and product survey. *Food Additives and Contaminants*, 24(Suppl. 1):91–107.

APPENDIX 1. DATA ON FURAN CONCENTRATIONS IN FOODS FROM 2004 TO 2009

a. Australia

Coffee type	Total number of samples	Furan concentration (µg/l)	
		Mean	Minimum–maximum
Cappuccino	10	32.3	23–50
Latte	10	22.9	7–49
Flat white	8	33.2	25–53
Long black	4	42.0	16–77
Short black	2	112.5	15–210
Mocha	2	23.5	12–35
Instant black	1	2.0	—
Instant white	2	2.5	2–3
Ground coffee	2	23.0	22–24

Source of analytical data: Food Standards Australia New Zealand (FSANZ, 2008).

b. Brazil

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Baby food	31	20	17.8	18.6	2.5	95.5
Canned feijoada (black beans)	2	0	0	2.4	—	—
Canned vegetables	3	0	0	2.4	—	—
Canned peach	2	0	0	2.4	—	—
Canned tuna	3	2	5.6	6.4	7.7	9.2
Canned tomato purée	1	0	0	2.4	—	—

Brazil (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Canned tomato sauce	3	0	0	2.4	—	—
Jam (strawberry)	1	0	0	2.4	—	—
Doce de leite (milk-based sweet)	2	1	1.9	3.1	—	—
Soya sauce	3	3	25.7	25.7	13.1	50.0
Roasted ground coffee (powder)	3	3	2998.1	2998.1	1946.4	5021.4
Bread	1	1	8.5	8.5	—	—

[a] All foods were analysed as bought. Two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero (trace = 0), and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ of 2.4 μg/kg (trace = LOQ). If all analysed samples within the same food group showed quantifiable results, the two mean values are similar. Not detected results are less than the LOD of 0.7 μg/kg. Minimum and maximum values were derived assuming only quantifiable results.

Source of analytical data: Arisseto, Vicente & Toledo (2009)

c. Canada

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Juice and drinks (cocktail)	3	13.1	6.7–16.7
Apple sauce	5	11.1	6.3–19.2
Mixed fruits	4	27.2	4.8–51.2
Peaches	2	17.7	13.4–21.9
Pineapples	3	4.8	3.4–6.7
Asparagus	4	5.5	2.8–11.4
Beans	15	63.3	18.2–195
Beets	4	100.4	11.7–338

Canada (contd)

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Carrots	5	43.9	26.9–70.9
Corn	5	36.1	28.1–52.1
Mushrooms	5	17.2	11.3–26.0
Peas	5	40.3	26.8–73.5
Potatoes	4	65.2	20.2–114
Tomatoes & pasta sauces	22	51.9	6.3–200
Tomato/vegetable juices (clamato, cocktail)	8	8.5	4.3–14.1
Other vegetables (artichoke, palm)	4	9.5	1.1–27
Condiments & sauces (e.g. ketchup, chili)	12	60.7	7.7–286
Baked beans	5	580.6	368–824
Pasta	5	396.4	151–1230
Peanut butter	1	10.6	—
Tuna	5	21.8	15.4–27.9
Salmon	5	13.4	9.3–18.1
Sardines	4	33.5	17.5–65.5
Shellfish	1	171	—
Soup (condensed cream of mushroom)	1	93.5	—
Chili con carne	5	386.4	241–863
Meat spreads	5	100.7	51–172
Meat (luncheon meat, flakes)	5	22.3	13.9–39
Stew & meatball	4	309	34.6–1030
Ground roasted coffee (powder)	1	4590	—
Instant coffee (powder)	2	413	279–547
Baby food	17	96.3	8.5–331

Source of analytical data: Becalski et al. (2009)

d. European Union

Food groups	Total number of samples	Furan concentration (μg/kg)[a,b]		
		Mean (lower–upper)	Minimum	Maximum
Instant coffee	48	588–589	0–8	2200
Roasted bean coffee	9	2271–2272	0–5	4895
Roasted ground coffee	66	1112–1114	0–5	5749
Coffee (non-specified)	275	1691	0–2	6500
Baby food	985	24–25	0–0.03	215
Infant formula	35	18–19	0–2	56
Baked beans	42	25–27	0–4	80
Beer	86	4–6	0–1	28
Cereal product	99	10–14	0–0.2	168
Fish	9	8–10	0–4	24
Fruit juice	203	4–6	0–0.5	420
Fruits	84	2–7	0–0.6	27
Meat product	65	19–22	0–2	115
Milk product	20	13–15	0–1	80
Sauces	207	8–12	0–0.1	120
Soups	198	23–24	0–0.7	225
Soya sauce	51	23–25	0–10	78
Vegetable juice	45	2–7	0–1	20
Vegetables	95	7–12	0–1	74
Other products	286	22–23	0–1	164

[a] With the exception of the maximum value, a range is provided when there was a difference between the estimated lower- and upper-bound furan concentrations. The lower-bound value was derived by assuming that values below the LOD and values between the LOD and the LOQ were set to zero, and the upper-bound value was derived by assuming that values below the LOD and values between the LOD and the LOQ were set to the LOD or the LOQ, respectively.

[b] Only 16% of the results in the EFSA database were obtained after preparation of the sample as consumed before the analysis.

Source of analytical data: EFSA (2009)

e. Japan

Food groups	Total number of samples	Furan concentration (μg/kg)	
		Mean	Minimum–maximum
Baby foods	15	22.2	1.4–90
Infant formula[a]	11	3.9–4.6	nd–36

nd, not detected

[a] For infant formula, two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero, and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ of 1 μg/kg.

Source of analytical data: Yoshida et al. (2007)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Fruit juices	26	24	2.7	2.7	1.1	5.4
Grapefruit juice	1	1	1.8	1.8	—	—
Apple juice	10	8	2.1	2.1	0.6	5.6
Grape juice	4	3	0.8	0.8	0.7	1.3
Pineapple juice	2	2	1.5	1.5	1.1	1.9
Orange juice	3	1	0.5	0.7	—	—
Dried fish	23	16	4.9	4.9	1.6	22
Canned vegetables	15	15	16	16	1.5	79
Canned boiled soya beans	10	10	29	29	7.4	54
Canned fruits	15	14	2.7	2.7	1.6	6.8
Canned cooked foods	15	15	69	69	11	140
Canned sauces	5	5	73	73	18	130
Canned soups	10	10	19	19	7.7	44
Retort cooked foods	100	100	42	42	4.5	140

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Retort ready-to-eat baby foods	40	40	30	30	8.7	86
Jarred infant cooked foods	35	35	21	21	3.6	55
Jarred infant cooked foods (fruits and vegetables)	5	5	43	43	5	140
Barley tea	3	3	17	17	4.4	30
Canned coffee	80	80	65	65	4.1	150
Canned brewed green tea	31	30	1.4	1.4	0.4	3.3
Soya sauces	30	30	40	40	16	100
Liquid seasonings made from soya sauce	20	20	45	45	19	100
Fermented soya bean pastes	30	30	34	34	2.0	290
Fermented soya bean pastes made with soya bean kouji	30	30	250	250	87	770
Fermented soya bean (i.e. natto)	30	0	0.0	0.4	—	—
Deep fried soya bean curds	30	1	0.0	1.0	—	—
Soya bean curds	10	5	1.1	1.1	1.4	2.3
Soya bean milks	10	2	0.3	0.5	0.6	2.4
Emulsified sauces	6	3	6.4	6.4	2.2	31
Other sauces	14	14	33	33	6.4	58
Canned fish	20	20	76	76	3.2	300
Salted and dried fishes	17	12	6.8	6.8	1.4	22

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)[a]			
			Mean (trace = 0)	Mean (trace = LOQ)	Minimum	Maximum
Steamed fish paste	20	1	0.065	0.48	—	—
Brewed barley tea	10	5	2.1	2.1	2.0	8.8
Brewed roasted tea	10	10	8.7	8.7	3.6	16
Brewed coffee	30	30	34	34	5.8	150
Japanese seasoning	10	10	8.8	8.8	4.3	13

[a] Two means are given in the table: the "lower bound" derived by assuming that not detected results and results between the LOD and the LOQ are zero (trace = 0), and the "upper bound" derived by assuming that not detected results and results between the LOD and the LOQ are equal to the LOQ (trace = LOQ). If not detected results and results between the LOD and the LOQ were not considered in calculations or all analysed samples within the same food group showed quantifiable results, the two mean values are similar. Canned cooked foods, canned soups and retort cooked foods were prepared according to the instructions on the package.

Source of analytical data: Japan Ministry of Agriculture, Forestry and Fisheries (MAFF, 2009a)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Median	Minimum–maximum
Fruit (can)	3	1	4.0	—
Vegetables (can)	3	3	8.0	4.0–47
Curry (can)	1	1	200	—
Stew (can)	1	1	58	—
Seasoned vegetables (can)	1	1	70	—
Meat sauce (can)	1	1	140	—
Soup (can)	2	2	36	25–47
Retort pouch curry (aluminium pouch)	10	10	46.6	30–110
Retort pouch stew (aluminium pouch)	1	1	16	—
Retort pouch hashed beef (aluminium pouch)	1	1	32	—

Japan (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Median	Minimum–maximum
Retort pouch soup (aluminium pouch)	2	2	22.5	20–25
Retort pouch pasta sauce (aluminium pouch)	4	4	47.5	10–100
Retort pouch rice bowl (aluminium pouch)	2	2	46.5	36–57
Infant and toddler foods (aluminium pouch)	8	8	16	9–57
Infant and toddler foods (glass bottle)	8	8	16	3–140
Infant and toddler drinks (glass bottle)	2	2	2.6	1.8–3.3
Infant and toddler drinks (PET bottle)	2	1	18	—
Coffee (can)	10	10	58.5	35–120
Coffee (PET bottle)	4	4	89.5	43–120
Coffee (carton)	2	2	61	39–83
Tea (can)	3	3	1.4	1.2–1.6
Tea (carton)	1	1	2.7	—
Tea (PET bottle)	2	1	1.3	—
Vegetable juice (can)	3	3	4.0	3.8–4.5
Vegetable juice (carton)	3	3	1.9	1.2–2.3
Soya sauce (glass bottle)	1	1	30	—
Soya sauce (PET bottle)	5	5	36	22–54
Noodle sauce (glass bottle)	2	2	67	40–94
Noodle sauce (PET bottle)	2	2	34.5	21–48
Soya bean paste (plastic container)	5	5	55	2–280
Soya bean paste (bag)	1	1	9.0	—
Emulsified sauce (plastic container)	1	0	—	—
Non-emulsified sauce (plastic container)	3	3	40	37–43

PET, polyethylene terephthalate
Source of analytical data: Japan Ministry of Agriculture, Forestry and Fisheries (MAFF, 2009b)

f. Republic of Korea

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Baby food (powdered milk)	47	29	3.8	1.0–20.7
Baby food (soup)	50	50	22.5	2.1–102.5
Baby food (beverages)	20	17	5.5	1.2–21
Sweet corn (canned, jarred)	9	9	8.6	2.1–23
Kidney bean (canned, jarred)	3	3	16.2	0.9–47.5
Bamboo shoot (canned, jarred)	2	2	2.1	0.9–3.6
Mushroom (canned, jarred)	3	3	11.4	2.3–30.9
Sesame leaf (canned, jarred)	3	3	44.1	23.1–58.3
Baby corn (canned, jarred)	1	1	5.7	5.6–5.8
Olive (canned, jarred)	6	6	3.3	1.3–6.3
Cucumber pickle (canned, jarred)	2	2	4.0	2.3–5.8
Caper (canned, jarred)	2	2	2.9	2.6–3.9
White peach (canned)	1	1	3.5	2.5–5.0
Mandarin (canned)	1	1	2.6	2.1–3.0
Mango (canned)	1	1	1.3	0.8–2.1
Cherry (canned)	1	1	4.0	2–8
Pork luncheon meat (canned)	12	12	9.2	2.8–17.8
Korean seasoned pork or beef (canned)	5	5	63.3	14–194
Chicken (canned)	2	2	17.7	15.3–22
Tuna (canned)	13	13	27.4	5.7–79.4
Mackerel (canned)	5	5	21.7	10.6–43.3
Saury (canned)	5	5	66.7	20.8–211.6
Whelk (canned)	4	4	38.6	28.4–60.6
Oyster (canned)	1	1	181.6	169.1–194.1
Crab (canned)	1	1	0.4	0.4–0.5

Republic of Korea (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Curry	10	7	10.7	7.7–15.1
Stir-fried bean paste	10	10	42.3	15.9–65.4
Instant soup	7	7	6.6	3.4–9.5
Thick beef soup	3	3	2.7	1.9–3.7
Soup (powder)	10	10	17.6	2.1–42.1
Soup (instant)	5	5	18.5	9.0–36.2
Soya sauce	5	5	16.3	1.5–28.4
Chili sauce	1	1	6.7	6.1–7.0
Pork rib sauce	1	1	12	10.–13.7
Pepper sauce	1	1	12.7	9.5–17.8
Oyster sauce	1	1	38.1	35.2–43.8
Spaghetti sauce	1	1	40.6	37.7–45.9
Jam (strawberry)	9	8	3.3	2.2–4.0
Jam (grape)	3	3	2.4	1.9–3.0
Jam (blueberry)	1	1	2.3	2.3–2.4
Jam (apple)	1	1	4.8	4.5–5.0
Jam (mixed fruits)	1	1	3.1	2.6–3.4
Bread	5	2	1.9	1.5–2.3
Biscuit	15	15	7.6	1.8–26.3
Snack	10	10	6.8	2.8–14.1
Orange juice (canned, jarred)	14	11	4.8	3.2–7.6
Grape juice (canned, jarred)	10	9	3.7	2.6–9.2
Pomegranate juice (canned, jarred)	2	2	3.6	2.7–4.6
Mango juice (canned, jarred)	2	2	3.3	2.8–3.8
Pear juice (canned, jarred)	1	1	3.0	2.9–3.0
Citrus juice (canned, jarred)	1	1	2.3	2.3–2.3

Republic of Korea (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum
Lemon juice (canned, jarred)	1	1	1.7	1.7–1.7
Apple juice (canned, jarred)	1	1	5.2	5.1–5.4
Aloe juice (canned, jarred)	1	1	5.5	5.4–5.7
Tomato juice (canned, jarred)	2	2	5.7	3.9–7.8
Japanese apricot juice (canned, jarred)	1	0	—	—
Mandarin juice (canned, jarred)	2	2	2.2	1.9–2.4
Mixture juice (canned, jarred)	2	2	3.0	1.9–4.3
Nutritional/diet drinks (vitamin)	4	4	7.4	4.9–10.1
Nutritional/diet drinks (red ginseng)	3	3	7.1	0.8–19.6
Nutritional/diet drinks (dietary fibre)	2	2	7.8	2.4–13.7
Nutritional/diet drinks (royal jelly)	1	1	6.0	3.5–7.4
Instant coffee (powder)	11	11	90.1	22.6–224.5
Instant coffee (liquid)	11	11	3.5	0.7–4.5
Coffee mix (powder)	10	10	54.2	26.4–99
Coffee mix (liquid)	10	10	3.6	1.1–5.5
Ground roasted coffee (powder)	10	10	814.1	267.1–2552.7
Ground roasted coffee (brewed)	4	4	48.5	30.7–67.1

Source of analytical data: Kim et al. (2009b)

g. Switzerland

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Baby food	101	101	21.7	1–153
Fruit and vegetable juices for babies	4	4	11.8	1–40
Canned or jarred vegetables	17	11	6.5	3–12
Canned fruits	2	1	6	—
Canned soup	2	2	31	19–43
Tins containing meat	2	2	9.0	4.0–14
“Sugo”, tomato and chili sauces	13	12	10.6	4–39
Soya sauce, hydrolysed vegetable protein	7	7	48.9	18–91
Vegetables, fresh	8	8	—	—
Vegetables, prepared	5	3	12.3	5.0–19
Bread, toast	39	30	46.4	5.0–193
Savory snacks	27	24	59	9–143
Sweet, pastry and biscuit	7	3	17.3	7.0–24
Sugar, caramel, pudding	15	11	312	4–1956
Dried fruits and nuts	16	1	7.0	—
Meat and meat products	5	1	10	—
Roasted coffee (brewed)	16	15	82.9	13–199
Roasted coffee (powder)	18	18	1979	22–5938
Instant coffee (brewed)	17	17	17	1–51.3
Instant coffee (powder)	11	11	783.3	44–2150
Green coffee	6	0	—	—
Coffee substitute (brewed)	4	4	6.8	1–15
Coffee substitute (powder)	4	4	586.3	102–1770
Dark beer	1	1	3	—
Cola soda pop	1	0	—	—

Switzerland (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Hot chocolate and malt beverage	2	0	—	—
Whole milk ultra-high temperature	1	0	—	—
Plum beverage	1	0	—	—
Beetroot juice with fruit juice	1	0	—	—
Potato flakes, not prepared	1	0	—	—
Instant gravy	1	0	—	—
Hash browns, not prepared	1	0	—	—
Milk chocolate	1	1	21	—
Spice spread	1	1	24	—
Dried French beans	1	1	5	—
“Eierflädli” (cut omelette for soups)	1	0	—	—
Liquorice	4	3	17.7	13–25
Burnt almonds	1	1	15	—
Flour and starch	5	0	—	—
Flour and starch (roasted)	5	5	1932.8	1050–3708
“Basler Mehlsuppe”, not prepared (powder)	1	1	5	—
Oxtail soup, not prepared (powder)	1	1	8	—
Compact black garnish paste	1	1	9	—

[a] Minimum and maximum values were derived assuming only quantifiable results.
Source of analytical data: Reinhard et al. (2004); SFOPH (2004)

Switzerland (contd)

Food groups	Total number of samples	Furan concentration (µg/kg)	
		Median	Minimum–maximum
Baby foods (jarred)	20	29	12–69
Baby foods (powder)	4	13	1.0–38
Honey	5	4	3.0–10
Coffee (extract)	4	98	73–125
Coffee (beans)	4	4400	2650–5050
Sweet corn (canned)	1	4	—
Peanuts (roasted, in honey)	1	4	—
Jam (apricot)	1	1	—

Source of analytical data: Kantonales Laboratorium Basel (2004)

h. United States of America

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Baby food (juices)	11	11	3.4	1.4–8.2
Baby food (canned/ jarred)	132	130	38.1	1.3–112
Baby food (cereal)	5	2	5.2	3.9–6.4
Infant formula	42	28	11.9	2.5–26.9
Roasted coffee (brewed)	8	8	46.7	33.6–84.2
Instant coffee (brewed)	6	2	6.0	4.8–7.2
Soup	36	36	36.6	6.7–125
Canned fish	9	6	5.6	1.5–8.1
Canned vegetables	49	46	21.9	0.8–85.6
Vegetables, fresh	4	1	2.1	—
Vegetable juices	7	7	5.3	3.2–7.6
Bread	12	0	—	—
Baked products	43	11	6.7	1.2–30.1

United States of America (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (μg/kg)	
			Mean of positives	Minimum–maximum[a]
Crackers and crispbread	4	4	12.2	4.2–18.6
Breakfast cereal	25	16	18.5	2.3–47.5
Meat products	33	20	6.2	0.3–39.2
Topping	5	5	3.5	1.5–6.6
Mayonnaise	3	0	—	—
Ketchup	1	1	12.3	—
Honey	2	2	9.6	8.3–10.4
Marshmallows	4	0	—	—
Syrup	7	6	22.9	0.5–88.3
Sugar	4	4	9.2	1.0–17.7
Candy bars	9	8	2.5	1.0–5.5
Nuts and nut butter	7	7	5.2	2.1–7.5
Desserts	12	11	4.7	1.5–13
Beverages (e.g. tea, soda)	14	9	0.7	0.4–1.4
Evaporated milk	3	3	12.5	10.9–15.3
Condensed milk	2	0	—	—
Whole milk	1	0	—	—
Cheese	6	5	1.3	0.4–2.9
Sweet-based milk	1	1	3.8	—
Jam, jelly and preserves	46	44	7.5	0.9–37.4
Gravies	8	8	47.8	13.3–173.6
Snacks	32	32	17.8	1.6–64.7
Liquid eggs	1	0	—	—
Fat and oils	2	1	5.4	—
Sauces	33	30	11.9	3.3–46
Ready-to-eat meals (e.g. pasta, chili)	12	12	45.4	9.5–94.4

United States of America (contd)

Food groups	Total number of samples	Number of positive samples	Furan concentration (µg/kg)	
			Mean of positives	Minimum–maximum[a]
Fruits (canned)	19	18	4.9	1.1–10.7
Fruit juices	35	21	4.8	0.5–30.5
Apple sauce	9	9	1.6	0.5–4.3
Dried fruits	8	7	1.3	0.6–2.2
Nutrition drinks	21	19	33.6	2.3–174
Beer	8	4	1.9	0.8–4.4
Chocolate drinks	10	4	4.1	0.5–10.3
Baked beans	15	15	60.4	23.3–122
Soya sauce	5	5	52.1	17.2–75.6

[a] Minimum and maximum values were derived assuming only quantifiable results.
Source of analytical data: USFDA (2009)