The MAK Collection for Occupational Health and Safety

Furan

MAK Value Documentation – Translation of the German version from 2006

A. Hartwig[1,*], MAK Commission[2,*]

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Geb. 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

* email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

Keywords: furan; carcinogenicity; toxicity; genotoxicity; bile duct; liver; haematopoietic system; mononuclear leukaemia

Citation Note: Hartwig A, MAK Commission. Furan. MAK Value Documentation – Translation of the German version from 2006. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2018 Jan;3(1):1-26]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb11000e4018_w

Republished (online): 12 Dec 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb11000e4018

Manuscript completed: 17 Feb 2005

Published (online): 24 Jan 2018

The commission established rules and measures to avoid conflicts of interest.

Furan / 1,4-Epoxy-1,3-butadiene

MAK Value Documentation

A. Hartwig[1, *], MAK Commission[2, *]

DOI: 10.1002/3527600418.mb11000e4018

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated all toxicological endpoints of furan [110-00-9].
Data indicate a genotoxic potential of furan especially due to the mutagenic metabolite *cis*-2-butene-1,4-dial. In carcinogenicity studies hepatocellular carcinomas were observed in rats and mice and cholangiocarcinomas in rats starting at the lowest tested dose of 2 mg/kg body weight and day with an incidence of at least 86%. A no observed adverse effect level (NOAEL) was not obtained. Therefore, furan is classified into Category 2 for carcinogens.
Furan is not classified into a Category for Germ Cell Mutagens as effects in testes and ovaries are only observed at doses that induce severe liver toxicity.
Calculations show that dermal absorption can contribute substantially to the systemic toxicity. Therefore, furan is designated with an "H".
There are no studies investigating the developmental toxicity and sensitizing potential of furan.

Keywords

furan; divinylene oxide; 1,4-epoxy-1,3-butadiene; furfuran; oxacyclopentadiene; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; genotoxicity; carcinogenicity; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

Author Information

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute for Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Geb. 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

[*] Email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

2

Furan

MAK value	**–**
Peak limitation	**–**
Absorption through the skin (2005)	**H**
Sensitization	**–**
Carcinogenicity (2005)	**Category 2**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	divinylene oxide, 1,4-epoxy-1,3-butadiene, furfuran, oxacyclopentadiene
Chemical name	1,4-epoxy-1,3-butadiene
CAS number	110-00-9
Structural formula	
Molecular formula	C_4H_4O
Molar mass	68.08 g/mol
Melting point	–85.6 °C (IARC 1995)
Boiling point at 1013 hPa	31.4 °C (IARC 1995)
Density at 20 °C	0.9514 g/cm³ (IARC 1995)
Vapour pressure at 25 °C	600 hPa (SRC 2005)
log K_{OW}[1)]	1.34 (IARC 1995)
1 ml/m³ (ppm) ≙ 2.825 mg/m³	**1 mg/m³ ≙ 0.354 ml/m³ (ppm)**

1) Octanol/water partition coefficient

1 Toxic Effects and Mode of Action

A 2-year study with rats and mice (gavage) revealed a high incidence of adenomas or carcinomas in the liver (>70%) in both species, bile duct carcinomas (incidence: >80%), damage to the haematopoietic system and leukaemia in rats, and phaeochromocytomas of the adrenal glands in mice even at the low dose of 2 mg/kg body weight and day (rats) and 8 mg/kg body weight and day (mice).

Furan is readily absorbed via the airways and, according to theoretical calculations, also through the skin. In humans, it has irritative effects on the eyes and respiratory tract, induces an urge to cough and may lead to oedema of the larynx and lungs.

No valid studies with repeated inhalation exposure are available. In animal studies, the main effects after single or repeated oral doses were cytotoxic effects in the liver and bile ducts followed by proliferation in the organs.

Some of the available in vitro and in vivo studies yielded evidence that furan causes clastogenic effects (in vitro: sister chromatid exchange (SCE) and chromosomal aberrations with CHO cells (a cell line derived from Chinese hamster ovary); in vivo: chromosomal aberrations in bone marrow). However, the results were not clearly positive with increasing doses. Furan was not mutagenic in several Salmonella typhimurium strains in the presence or absence of a metabolic activation system. However, the metabolite cis-2-butene-1,4-dial was found to have mutagenic potential in the aldehyde-sensitive Salmonella strain TA104 and formed diastereomeric adducts with the nucleotides of cytosine, guanine and adenine. The mutation pattern observed in activated oncogenes of furan-induced liver tumours in mice differed from that observed in spontaneous tumours.

If the metabolism is not saturated, furan is completely converted within 4 hours; in the case of saturated metabolism, it is only partially converted to the cis-2-butene-1,4-dial dialdehyde via cytochrome P450 and then to CO_2, for example, presumably via maleic acid and fumaric acid.

Furan does not have toxic effects if metabolic activation is inhibited by a cytochrome P450 inhibitor. Therefore, the toxic effects are not caused by furan itself, but by a metabolite, probably cis-2-butene-1,4-dial.

Penetration of the skin is assumed from calculations made on the basis of the physicochemical properties of furan.

There are no studies available of the reproductive toxicity, sensitizing effects or germ cell mutagenicity of furan.

2 Mechanism of Action

Exposure to furan induced inflammation, apoptosis and necrosis in the liver, followed by the pronounced regenerative proliferation of hepatocytes (Goldsworthy and Foley 2001). Depending on the dose and duration of exposure, new bile duct cells and metaplasia develop, preferably in the right and caudate liver lobes. After several weeks of proliferation, fibrosis develops in the bile ducts forming goblet cells, Paneth cells (bactericidal and immunological functions), neuroendocrine cells characteristic of the small intestine and ductular hepatocytes (Elmore and Sirica 1993;

Sell 2002). Long-term exposure of rats induced necrosis and tumours mainly in the liver (preferably in the right and caudate liver lobes) and bile ducts, lesions in the haematopoietic system and mononuclear leukaemia. Hepatocellular adenomas and carcinomas and adrenal phaeochromocytomas were observed in mice (NTP 1993). Kedderis and Ploch (1999) noted that the furan concentrations that were used in this study and that induced the above-described effects and pronounced cell proliferation were cytotoxic and they regarded furan as a cytotoxic carcinogen with a "threshold" (Kedderis and Ploch 1999). However, "threshold" studies were carried out only for hepatotoxicity in female mice and yielded a NOAEL (no observed adverse effect level) of 2 mg/kg body weight and day (Goldsworthy and Foley 2001). Details of the findings have not yet been published. The study was deliberately carried out only with female mice because liver tumours, but no bile duct carcinomas, were found in mice in the NTP study (NTP 1993) and female B6C3F1 mice have a lower incidence of spontaneous liver tumours. Therefore, the NOAEL of 2 mg/kg body weight and day refers only to hepatotoxicity in mice. Rats have been found to be more sensitive because in the NTP study doses as low as 2 mg/kg body weight and day induced liver adenomas or carcinomas in 10% of the males and 4% of the females and bile duct tumours in 86% of the males and 98% of the females (NTP 1993). As lower doses were not included in any of the available studies in rats, it is not possible to draw conclusions about a possible dose without effects in rats.

Metabolically activated furan induced the uncoupling of oxidative phosphorylation in the mitochondria of hepatocytes in vitro and in vivo (Mugford et al. 1997). The authors assumed that the uncoupling reduces the supply of ATP to the ion pumps and is involved in a loss in the ability to maintain the calcium gradient, which leads to an increase in the calcium level in the cell. This is assumed to lead to the activation of calcium-dependent proteases, phosholipases and endonucleases (Nicotera et al. 1990). However, recent studies have shown that uncouplers of the mitochondrial respiratory chain induce apoptosis by opening the mitochondrial transition pores rather than via the described calcium mechanism (Klöhn et al. 2003). Unspecific reactions cause necrosis only at higher doses (8 mg/kg body weight and day and above in female B6C3F1 mice; Fransson-Steen et al. 1997).

An irreversible concentration-dependent and time-dependent decrease in ATP in the cells was detected after the exposure of isolated rat hepatocytes to furan. ATP depletion was found at a furan concentration of 100 μM, but not at 2 or 10 μM. This depletion in ATP, which was observed in vitro and in vivo and results from the uncoupling of oxidative phosphorylation, is amplified by the activation of the ATPase activity and leads to cell death. The decrease in the ATP concentration can be prevented by 1-phenylimidazole if cytochrome P450 is inhibited. The same effect can be achieved with fructose and oligomycin. Fructose protects the cells from chemically induced damage to the mitochondria by increasing glycolytical ATP production, while oligomycin inhibits the F1F0-ATPase activity and thus the degradation of ATP produced glycolytically. Furan itself has no effect in these in vitro experiments if the cytochrome activity is inhibited; this substantiates the role of metabolic activation and supports the mechanism via ATPase (Mugford et al. 1997). The dialdehyde cis-2-butene-1,4-dial is probably the effective metabolite (Chen et al. 1995). It is very reactive and rapidly reacts with thiol groups, for example with GSH (glutathione;

Peterson et al. 2000), or with the exocyclic and endocyclic nitrogen atoms of the deoxyribonucleotides of cytosine, guanine and adenine (Byrns et al. 2002). According to Mugford et al. (1997), the reaction products may induce the degradation of macromolecules in the cells and damage to the cell membrane. Most commonly, this results in necrosis in the liver and, to a small extent, apoptosis (Kedderis and Ploch 1999). Apoptosis is an ATP-dependent process. Therefore, it is assumed that low furan concentrations produce apoptosis and high concentrations lead to necrosis. Fumaric acid and maleic acid are presumably major products on the metabolic pathway to CO_2 and might belong to the group of uncouplers, which, like a number of peroxisome proliferators, transfer protons through the inner membrane in the form of lipophilic acids or act via the protein-mediated uncoupling of free fatty acids (Keller et al. 1992).

As hepatocytes have a large ATP pool and can survive cellular ATP losses of up to 95%, it is unlikely that these effects caused the high incidence of various types of tumours in rats and mice (Mugford et al. 1997).

It is therefore assumed that also the faulty repair of DNA breaks contributes to the hepatotoxicity of furan. DNA double strand breaks were found in isolated rat hepatocytes as early as 30 minutes after the beginning of exposure; in the surviving cells, they were repaired after 24 hours. When an endonuclease inhibitor was added, the number of DNA double strand breaks decreased considerably (Kedderis and Ploch 1999).

Irrespective of these possible genotoxic effects, the findings obtained to date suggest that furan has mainly tumour-promoting properties. These are based on the uncoupling of oxidative phosphorylation (or other cytotoxic effects), cell elimination and the induction of an increase in regenerative proliferation. The development of fibrosis suggests that, as in the case of 2-acetylaminofluorene, cell replacement is disturbed by oval cell proliferation and differentiation is increased with a tendency for different, primarily fibre-producing bile duct cells, which leads to a conversion of the hepatic cords and promotes the growth of initiated cells (Bitsch et al. 1999, 2000).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution and elimination

In inhalation studies with different ventilation rates and exposure concentrations of 400 to 600 mg/m^3 (142 to 212 ml/m^3) carried out in 9 to 12 mongrel dogs of both sexes, the retention of furan was found to be greater than 90% (Egle and Gochberg 1979).

When male Fischer 344 rats were given [2,5-^{14}C]-furan as a single gavage dose of 8 mg/kg body weight, furan was absorbed rapidly and 14% was exhaled unchanged within 24 hours, 11% of it within the first hour. 26% of the radioactivity was exhaled as $^{14}CO_2$, 20% of the radioactivity was found in the urine and 22% in the faeces. After 24 hours, 68% of the 19% that remained in the body (13% of the administered dose) was detected in the liver and smaller amounts were found in the kidneys and gastrointestinal tract. It was possible to extract 20% of the radioactivity in the liver with

organic solvents, while the remainder was bound, but not to liver DNA. In the rat, the fraction in the liver that was not bound was eliminated according to pseudo first-order kinetics with a half-time of 1.8 days, while elimination from the blood and kidneys was more complex. When a [2,5-^{14}C]-furan dose of 8 mg/kg body weight and day (gavage) was given 8 times, elimination with the urine increased (33%). The concentration of the radioactivity was increased 6-fold in the blood and kidneys and 4-fold in the liver (Burka et al. 1991).

Another publication reported that following a single oral dose of furan, metabolism and elimination from the liver was complete in vivo after 4 hours according to model calculations that were checked against in vivo data. In rat hepatocyte suspensions, the metabolism of furan was complete after 4 hours at low concentrations (10 µM), whereas high furan concentrations (100 µM) induced cytotoxicity and GSH depletion (Carfagna et al. 1993). If these results are converted to the doses used in the 2-year NTP study in rats (NTP 1993; see Section 5.7), furan concentrations that are cytotoxic in the liver in vitro would be reached at the middle NTP dose of 4 mg/kg body weight and day and above. A NOAEC (no observed adverse effect concentration) of about 1 µM furan was calculated from the in vivo data for DNA lesions and cell death in the liver. This would correspond to a furan dose of about 1 mg/kg body weight and day in rats (Carfagna et al. 1993). However, in the NTP study in rats (50 animals per dose group), bile duct carcinomas occurred in > 86% of the animals even at the low furan dose of 2 mg/kg body weight and day and liver adenomas and carcinomas were observed in > 4% of the animals. Therefore, it seems questionable whether the calculated NOAEL can be confirmed in rats in vivo.

A maximum reaction rate V_{max} of 27 µmol (1800 µg)/hour based on a rat weighing 250 g and a half maximum reaction rate K_M of 2 µmol (136 µg)/l were determined from the furan concentrations in the blood and liver of male Fischer 344 rats during 4-hour exposure to furan at 52, 107 or 208 ml/m³ (purity: > 99%). The constants determined with rat hepatocytes in vitro were: V_{max} = 0.02 µmol/hour and 10^6 cells and K_M = 0.4 µmol/l per 10^6 cells. If this is converted to a rat weighing 250 g and 128 × 10^6 hepatocytes/g liver, this corresponds to a V_{max} of 23 µmol/hour; this means that the parameters determined in vitro and in vivo are almost identical (Kedderis et al. 1993).

The pharmacokinetics of furan in rats in vivo was well simulated by incorporating the enzyme kinetic parameters determined in isolated rat hepatocytes into a pharmacokinetic model (Kedderis et al. 1993). If the amount of furan that would be transported to the liver after 4 hours of theoretical exposure to furan concentrations of 300 ml/m³ is compared with the amount of furan oxidized by hepatocytes, it is apparent that the rate of oxidation in human hepatocytes is about 37 times more rapid than the rate of supply with the blood. The rates of oxidation were 13 times greater and 24 times greater in rats and mice, respectively, than the rate of supply with the blood. This means that the metabolism is not saturated at 300 ml/m³ and enzyme induction cannot increase V_{max}. Moreover, a model calculation for humans showed that after 4-hour exposure to 10 ml/m³, humans absorb 3 and 10 times less furan than rats and mice, respectively (Kedderis and Held 1996). According to these calculations, the internal exposure to furan or its metabolites is lower for humans than for rats or mice at the same exposure concentration.

There are no data available for the skin penetration or dermal toxicity of furan. Based on an aqueous solution saturated with furan, a dermal flux of 0.759 mg cm^{-2} h^{-1} is calculated with the model of Fiserova-Bergerova et al. (1990) and of 0.063 mg cm^{-2} h^{-1} with the model of Guy and Potts (1993). This would correspond to the total absorption of 1519 mg and 125 mg, respectively, after the exposure of both hands and forearms (about 2000 cm^2).

3.2 Metabolism

When furan was given orally to male Fischer 344 rats, CO_2 and at least ten unidentified substances were observed as metabolites in the urine. The metabolism pattern after a single dose did not differ from that after multiple doses (Burka et al. 1991).

After furan is absorbed, it is bioactivated by cytochrome P450 and oxygen in the liver to form cis-2-butene-1,4-dial, which is regarded as the actual toxic agent (Chen et al. 1995, 1997; Kedderis and Ploch 1999) because there was a positive correlation between covalently bound metabolites and necrosis in the liver and kidneys (Masuda et al. 1984). The P450 2E1 iso form is mainly responsible for activation (Carfagna et al. 1993; Kedderis and Held 1996). The dialdehyde is presumably oxidized directly to maleic acid or fumaric acid after isomerization. Both acids are subject to rapid metabolism (Burka et al. 1991). Furan does not have toxic effects if the metabolic activation of furan is inhibited by a P450 inhibitor (Kedderis and Ploch 1999; Mugford et al. 1997).

When liver microsomes of Fischer 344 rats were incubated with [2,5-^{14}C]-furan in the presence of NADPH, the radioactivity was covalently bound to microsomal protein. In the absence of NADPH, binding to microsomal protein was reduced. Binding increased following the pretreatment of rats with phenobarbital, imidazole or pyrazole, but was reduced after treatment with β-naphthoflavone. If semi-carbazide, N-acetylcysteine or glutathione were added, furan or its metabolites were more likely to react with the free amino and thiol groups of the added substances than with microsomal protein (Parmar and Burka 1993). Another study carried out with microsomes of male F344 rats and the furan metabolite cis-2-butene-1,4-dial showed that the metabolite rapidly formed adducts with amino acids and glutathione. In this case, cis-2-butene-1,4-dial cross-linked 2 molecules of glutathione (Chen et al. 1997). A comparison of the rate of binding of cis-2-butene-1,4-dial to deoxyribonucleosides at pH 6.5 yielded the following sequence: dCyt » dGuo ~ dAdo. This sequence and the rate of binding were similar to those of formaldehyde with the same deoxyribonucleosides at pH 6.5 (Byrns et al. 2002).

4 Effects in Humans

Furan is readily absorbed via the airways and, according to theoretical calculations, also through the skin. In humans, it has irritative effects on the eyes and respiratory tract, induces an urge to cough and may lead to oedema of the larynx and lungs (Römpp 1999). There are no other data available for effects in humans.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The LC_{50} for furan after inhalation exposure for 1 hour was 3398 ml/m³ in rats and 42 ml/m³ in mice. Dyspnoea and pulmonary oedema were observed in the animals (NIOSH 2002).

5.1.2 Oral administration

In dogs and rabbits, the lowest observed lethal dose of furan was 234 mg/kg body weight after oral administration (NIOSH 2002).

Liver damage and hepatocyte proliferation were found in male B6C3F1/CrIBR mice and Fischer 344/CrIBR rats after single oral furan doses of 30 mg/kg body weight in rats and 50 mg/kg body weight in mice were given to 5 animals per group. The animals were examined 12, 24 or 48 hours and 4 or 8 days after the administration of furan. Even the first examination, 12 hours after the administration of furan, revealed midzonal degeneration and necrosis in the liver; the maximum extent was reached 24 hours after administration. Hepatocytes close to the central vein were affected the most. After 48 hours, midzonal inflammation and pronounced proliferation was observed, but hardly any necrosis. The liver had almost completely recovered after 8 days. The animals were injected intraperitoneally with [³H-methyl] thymidine 2 hours before the examination to establish the labelling index. The labelling index had increased considerably in mice and rats after 48 hours. At other times, there was no significant change in the labelling index compared with that in the control animals. According to the authors, this pattern of a maximum increase in compensatory proliferation 48 hours after administration is typical of hepatotoxic substances. Marked increases in plasma aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase activities were observed between 12 and 24 hours after the administration of furan. The liver and enzyme concentrations were almost normal again 8 days after the administration of furan (Wilson et al. 1992).

5.1.3 Dermal application

There are no data available.

5.1.4 Intraperitoneal and intravenous injection

After intraperitoneal injection, the LD_{50} for furan was 5.2 mg/kg body weight in rats and 6.9 mg/kg body weight in mice (Egle and Gochberg 1979). After intravenous injection, the lowest observed lethal dose in dogs was 140 mg/kg body weight (NIOSH 2002).

Groups of 10 male Swiss albino mice were given single intraperitoneal furan doses of 300 mg/kg body weight with and without pretreatment with the cytochrome P450 inhibitor piperonyl butoxide. In the pretreated animals centrilobular hepatic necrosis was less prevalent and, unlike in the animals that were not pretreated, proximal tubular necrosis of the outer renal cortex was not observed (IARC 1995).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

The exposure of rats to furan doses of 5 mg/m^3 for 4 hours per day for 26 weeks induced damage to the CNS and an increase in blood pressure (NIOSH 2002). When rats were exposed to furan at 200 mg/m^3 for 4 hours per day for 60 days, mortality was increased, structural and functional changes were observed in the trachea and bronchi, and liver damage was found (NIOSH 2002). After the exposure of rats to furan at 500 mg/m^3 for 2 hours per day, increased mortality and liver damage were observed after 30 days, and body weight gains were reduced, mortality was increased and jaundice was found after 9 weeks (NIOSH 2002).

Original data or an accurate study description were not available for any of these Russian studies dating from 1967 and 1968, and their validity could thus not be checked. Therefore, these studies have not been included in the evaluation.

5.2.2 Oral administration

Studies with repeated oral administration of furan are shown in Table 1.

A 16-day study and a 13-week study were carried out as range-finding studies for a 2-year NTP study with B6C3F mice and Fischer 344/N rats with gavage administration of furan (purity: > 99%) in corn oil.

In the 16-day study, all rats died at 80 mg/kg body weight and day and above during the first 8 days. In mice, 3 of 5 males died at 40 mg/kg body weight and day, 1 female survived at 80 mg/kg body weight and day and no animal survived at 160 mg/kg body weight and day. The exposed animals were very lethargic. In rats, enlarged and blotchy livers were observed in males at 20 mg/kg body weight and day and above and in females at 40 mg/kg body weight and day. In mice, examination of the organs did not reveal any findings that could definitely be attributed to furan (NTP 1993). The results are described in Table 1.

Table 1 Effects of furan after repeated oral administration

Species, strain, number of animals, sex/group	Exposure gavage	Findings	References
rat, Fischer 344/N, 14 ♂	**10–14 days**, 60 mg/kg body weight and day	atrophy of the right liver lobe, new bile duct tissue	Sirica et al. 1994
rat, Fischer 344/N, 5 ♂ per group	**1–2 weeks,** 0, 60 mg/kg body weight and day	development of "ductular hepatocytes" and bile duct epithelial cells in the liver	Elmore and Sirica 1991
rat, Fischer 344/N, 5 ♂ per group 5 ♀ per group	**16 days**, 0, 5, 10, 20, 40, 80 mg/kg body weight and day; 0, 10, 20, 40, 80, 160 mg/kg body weight and day	at necropsy: mottled and enlarged livers no histopathology or clinical pathology **20 mg/kg body weight and above**: ♂: body weight gains ↓ **40 mg/kg body weight**: ♀: body weight gains ↓ **80 mg/kg body weight and above**: all animals died within 8 days	NTP 1993
rat, Fischer 344/N, 5 ♂ per group	**3 weeks**, 0, 15, 30, 45 mg/kg body weight and day	distribution of bile duct fibrosis: **30 mg/kg body weight**: middle (5%) and caudate (22%) liver lobes **45 mg/kg body weight**: right (3%), middle (12%) and caudate (95%) liver lobes	Elmore and Sirica 1991
rat, Fischer 344/N, no other details	**5–6 weeks**, 0, 45 mg/kg body weight and day	after bile duct ligation: **0 mg/kg body weight**: 2% new bile duct tissue **45 mg/kg body weight**: 73% new bile duct tissue; γ-glutamyl transpeptidase activity ↑ after sham operation: 11% new bile duct tissue	IARC 1995
rat, Fischer 344/N, 6 ♂ per group	**1, 3, 6 weeks**, 8 mg/kg body weight and day	6 days before analysis: implantation of an osmotic pump containing [^{3}H] thymidine **week 1**: infiltration of inflammatory cells on visceral surfaces; labelling index: ♂ 3%, ♀ 12%	Wilson et al. 1992

Table 1 (continued)

Species, strain, number of animals, sex/group	Exposure gavage	Findings	References
		week 3: lesions on visceral surfaces; hypereosinophilic hepatocytes; hyperplasia with adjacent fibrosis of bile ducts; proliferation of bile ducts; labelling index: ♂ 9%, ♀ 9% **week 6**: bile duct fibrosis, in some cases with hyperplasia and metaplasia; labelling index: ♂ 7%, ♀ 14%	
rat, Fischer 344/N, 10 ♂/10 ♀ per group	**13 weeks**, 0, 4, 8, 15, 30, 60 mg/kg body weight and day	no clinical pathology ♂: **4 mg/kg body weight and above**: hyperplasia in the biliary tract ↑; bile duct fibrosis ↑; pigmentation of Kupffer's cells ↑ **8 mg/kg body weight and above**: degeneration of hepatocytes **15 mg/kg body weight**: relative kidney weights ↑ **15 mg/kg body weight and above**: body weight gains ↓; cytomegaly and necrosis of hepatocytes ↑ **30 mg/kg body weight**: relative and absolute liver weights ↑; relative kidney weights ↑; absolute thymus weights ↓ **30 mg/kg body weight and above**: nodular hyperplasia in the hepatocytes; dilation of renal tubules **60 mg/kg body weight**: mortality 9/10; atrophy of testes and thymus; necrosis of renal tubules ↑ ♀: **4 mg/kg body weight and above**: hyperplasia in the biliary tract ↑; pigmentation of Kupffer's cells **8 mg/kg body weight and above**: relative and absolute liver weights ↑; bile duct fibrosis ↑; pigmentation of Kupffer's cells ↑ **15 mg/kg body weight and above**: relative and absolute kidney weights ↑; cytomegaly, degeneration and necrosis of hepatocytes	NTP 1993

Table 1 (continued)

Species, strain, number of animals, sex/group	Exposure gavage	Findings	References
		30 mg/kg body weight and above: relative and absolute kidney weights ↑; nodular hyperplasia in hepatocytes ↑ **60 mg/kg body weight**: mortality 4/10; body weight gains ↓; absolute thymus weights ↓; dilation and necrosis in renal tubules ↑; atrophy of ovaries ↑	
mouse, B6C3F1, 5 ♂/5 ♀ per group	**16 days**, 0, 10, 20, 40, 80, 160 mg/kg body weight and day	no histopathology or clinical pathology **10 mg/kg body weight**: ♂: body weights ↑ **20 mg/kg body weight**: ♂: body weights ↑ **40 mg/kg body weight**: 3 ♂ died **80 mg/kg body weight**: 4 ♀ and 5 ♂ died **160 mg/kg body weight**: all animals died	NTP 1993
mouse, B6C3F1, 6 ♂ per group	**1, 3, 6 weeks**, 15 mg/kg body weight and day	6 days before analysis: implantation of an osmotic pump containing [^{3}H] thymidine **week 1**: some inflammatory cells on visceral surfaces; 25% labelling index **week 3**: necrosis and inflammation in parenchyma of visceral surfaces; 12% labelling index **week 6**: centrilobular pattern of vacuolated hepatocytes; 3% labelling index	Wilson et al. 1992

Table 1 (continued)

Species, strain, number of animals, sex/group	Exposure gavage	Findings	References
mouse, B6C3F1, 10 ♂/10 ♀ per group	**13 weeks**, 0, 2, 4, 8, 15, 30 mg/kg body weight and day, ♀ also 60 mg/kg body weight and day	no clinical pathology ♂: **4 mg/kg body weight**: 1/10 died **8 mg/kg body weight**: 1/10 necrosis of hepatocytes **15 mg/kg body weight**: necrosis and degeneration of hepatocytes: 1/10 each **15 mg/kg body weight and above**: relative and absolute liver weights ↑ **30 mg/kg body weight**: cytomegaly ↑; degeneration and necrosis of hepatocytes ↑; 2/10 hyperplasia in the biliary tract; 3/10 pigmentation of Kupffer's cells ♀: **15 mg/kg body weight**: degeneration of hepatocytes 3/10 **30 mg/kg body weight**: 1/10 died **30 mg/kg body weight and above**: relative and absolute liver weights ↑; cytomegaly ↑; degeneration and necrosis of hepatocytes ↑; hyperplasia in the biliary tract ↑; bile duct fibrosis ↑; pigmentation of Kupffer's cells ↑	NTP 1993

see Section 5.2.2 for results of the 2-year study (NTP 1993)

After 13-week exposure to furan doses of 0, 4, 8, 15, 30 or 60 mg/kg body weight and day, 9 of 10 male rats and 4 of 10 female rats of the high dose group died (see Table 1). The authors concluded that the changes in organ weights were secondary effects of the reduced body weight gains; these sometimes affected the relative organ weights and sometimes the absolute organ weights, at times to a statistically significant degree, but were never dose-dependent. In the livers of at least 8 of 10 animals in the high exposure group, cholangiofibrosis and hyperplasia in the biliary tract (in some cases, there was a statistically significant increase at doses as low as 4 mg/kg body weight and day), degeneration, cytomegaly and necrosis of the hepatocytes (statistically significant at 15 mg/kg body weight and day and above) were observed. Furthermore, dilated renal tubules with a necrotic epithelium and atrophy of the thymus and testes or ovaries were detected in the high dose group. Similar liver findings were observed in mice at 8 mg/kg body weight and day and above. In this study, the NOAEL was 4 mg/kg body weight and day for mice, while in rats adverse effects occurred in all dose groups (NTP 1993).

In addition, a stop-exposure study was carried out with 50 male rats that were given furan doses of 0 or 30 mg/kg body weight and day for 13 weeks and then observed for 21 months (see Section 5.7 and Table 2). A total of 6 rats died between the examinations after 9 and 15 months, while 14 rats died after month 15 and before the end of the 2 years. At the end of the 13-week exposure period, non-neoplastic changes were found at many sites, including fibrosis and hyperplasia in the bile ducts, cytomegaly, degeneration, hyperplasia, necrosis, cytoplasmic vacuolation in the hepatocytes and pigmentation of Kupffer's cells. The examinations after 9 and 15 months revealed an increase in the severity of these lesions as well as extensive chronic inflammation and a large number of cysts in the bile ducts. These findings were observed in all 10 of the animals that were examined from each group (NTP 1993).

The carcinogenic effects observed in the 2-year study are described in Table 2 in Section 5.7. Non-neoplastic changes of the liver were observed in male and female rats and mice of all dose groups, in most cases at incidences higher than 60%; they included cytomegaly, cytoplasmic vacuolation, focal degeneration, hyperplasia, necrosis and pigmentation. Chronic focal inflammation, cysts, metaplasia, focal fibrosis and hyperplasia were present in the biliary tract. Non-neoplastic lesions were found in rats at different sites of the haematopoietic system: hyperplasia in the bone marrow and ectasia in the pancreatic and mediastinal lymph nodes. In the males, also hyperplasia in the lymph nodes and congestion and cell proliferation in the spleen were found. Interim examinations carried out in additional rats after 9 or 15 months revealed a significant increase in the incidence of neoplastic and non-neoplastic lesions in the hepatocytes and bile ducts at the low concentration of 2 mg/kg body weight and day and above (10/10 animals in some cases) (NTP 1993).

Liver damage and hepatocyte proliferation were detected in male B6C3F1/CrIBR mice and Fischer 344/CrIBR rats after gavage treatment for 1, 3 or 6 weeks with furan doses of 8 mg/kg body weight and day in rats and 15 mg/kg body weight and day in mice. The animals were given [^{3}H-methyl] thymidine via an osmotic pump 6 days before the examination to establish the labelling index. After the administration of furan for 6 weeks, hyperplasia and metaplasia of fibrous tissue were found in the bile

ducts of rats of both sexes. In mice, no bile duct proliferation was observed, but there was increased centrilobular vacuolation in the liver. The labelling index was increased in rats and mice (Wilson et al. 1992).

After the exposure of male Fischer 344 rats to furan doses of 0, 15, 30, 45 or 60 mg/kg body weight and day for 2 or 3 weeks, bile duct fibrosis was observed at 30 mg/kg body weight and above. This occurred mainly in the middle and caudate liver lobes and was characterized by intestinal mucosal cells (goblet, Paneth and neuroendocrine cells) (Elmore and Sirica 1991).

When male Fischer 344 rats were given furan doses of 45 mg/kg body weight and day for 5 to 6 weeks beginning 1 week after bile duct ligation, about 73% of the liver was replaced by new, well-differentiated hyperplastic bile ducts in the treated animals. In addition to the synergism between furan administration and bile duct ligation, the γ-glutamyl transpeptidase activities were increased in the liver homogenates of these treated animals. In control animals, which were ligated in the same way and treated with corn oil, only about 2% of the liver consisted of new bile duct tissue; after a sham operation and treatment with furan about 11% new bile duct tissue formed in the liver (IARC 1995).

Groups of male Fischer 344 rats were given furan doses of 45 mg/kg body weight and day for 1, 3, 5, 7, 9, 12, 16 or 32 days. Hepatocellular necrosis was detected in the lower right liver lobes of the animals that underwent only short-term exposure. This was followed by inflammatory cell infiltration, bile duct hyperplasia and the development of metaplastic cells with the increasing exposure duration and bile duct fibrosis by day 32 (IARC 1995).

A furan dose of 60 mg/kg body weight and day given by gavage to 14 male Fischer 344 rats for 10 to 14 days induced marked atrophy of the right liver lobe. The bile duct structures that developed in this region were biliary epithelial cells and other cells characteristic of bile ducts in various stages of development (Sirica et al. 1994).

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

There are no data available.

5.4 Allergenic effects

There are no data available.

5.5 Reproductive and developmental toxicity

There are no data available.

5.6 Genotoxicity

5.6.1 In vitro

Furan was not mutagenic in bacterial mutagenicity tests with the Salmonella strains TA98, TA100, TA1535 or TA1537 up to doses of 3333 µg/plate. An S9 mix from rat and hamster livers was used after previous activation by Aroclor 1254 as metabolic activation system (Mortelmans et al. 1986). The metabolite cis-2-butene-1,4-dial formed by the biological activation of furan was mutagenic in the aldehyde sensitive Salmonella strain TA104; the mutagenic activity was concentration-dependent. There was no evidence of a mutagenic potential of cis-2-butene-1,4-dial in the Salmonella strains TA97, TA98, TA100 or TA102 at non-toxic concentrations. If GSH was added to the cultures together with furan, the number of revertants was reduced (Salmonella strain not specified). Pre-incubation of cis-2-butene-1,4-dial with GSH and subsequent addition to the cultures induced bis-GSH conjugates of the substance, and no mutagenicity occurred (Peterson et al. 2000). In the SCE test with CHO cells, there was a slight dose-dependent increase in SCE in the presence and absence of a metabolic activation system. At furan concentrations of 1.6 to 160 µg/ml, in the absence of a metabolic activation system the incidence of SCE increased by more than 20% compared with that in the control group; in the presence of a metabolic activation system an increase of more than 20% was observed only at the high concentration of 500 µg/ml (NTP 1993). According to the authors, the tests were carried out up to cytotoxic concentrations, but there are no exact data for toxicity. The test result must be regarded as "inconclusive" because the incidence of SCE was higher without metabolic activation than with metabolic activation.

In an in vitro UDS assay with primary rat and mouse hepatocytes, furan did not induce DNA repair synthesis at concentrations of up to 10 mM. Although furan exhibited high volatility in the medium, the authors assumed adequate exposure of the target cells on the basis of control determinations. However, they consider the results to be of limited validity because of the volatility of furan (Wilson et al. 1992). The publication did not present the original data of the UDS assay in detail.

In an in vitro chromosomal aberration test with CHO cells, furan (100 to 200 mM) induced structural aberrations in the presence of a metabolic activation system from rat livers (induced by Aroclor 1254). Aberrations were not induced in the absence of a metabolic activation system. There was no increase in aberrations if NADP was not included in the metabolic activation system (Stich et al. 1981).

In another in vitro chromosomal aberration test with CHO cells, a dose-dependent increase was observed in the presence and absence of a metabolic activation system. The number of aberrations was significantly increased at furan concentrations of 100 to 500 µg/ml in the absence of a metabolic activation system and at 500 or 1000 µg/ml in the presence of a metabolic activation system (NTP 1993).

Furan caused mutations in the $TK^{+/-}$ gene mutation assay with L5178Y mouse lymphoma cells in the absence of a metabolic activation system. One test yielded negative results and the second positive results at furan concentrations of 2600 µg/ml and above; the third test resulted in a significant increase in the mutant frequency at 1139 µg/ml. Positive effects were determined in the high dose range under condi-

tions where the relative growth (RTG) was reduced by 20% to 60% (McGregor et al. 1988; NTP 1993). An increase in the mutant frequency in the cytotoxic range is not regarded as a genotoxic effect. This study has not been included in the evaluation because of inconsistent results and no differentiation between large and small colonies.

In in vitro tests, the furan metabolite cis-2-butene-1,4-dial formed adducts with the 2′-deoxyribonucleosides cytidine, guanosine and adenosine, but not with thymidine (Byrns et al. 2002). This might be evidence of a genotoxic mechanism in tumour induction.

5.6.2 In vivo

In the Drosophila test for X-chromosomal recessive lethal mutations, furan yielded negative results in feeding studies or when given by abdominal injection (IARC 1995).

In the UDS assay, furan did not induce repair synthesis in mouse hepatocytes after single oral (gavage) doses of 0, 10, 50 or 100 mg/kg body weight (time of preparation: 12 hours after treatment) or of 0, 10, 50 or 200 mg/kg body weight (time of preparation: 2 hours after treatment). Likewise, repair synthesis was not induced in rat hepatocytes after single oral (gavage) doses of 0, 5, 30 or 100 mg/kg body weight (time of preparation: 2 or 12 hours after treatment) (Wilson et al. 1992).

The incidence of SCE was not increased in the bone marrow of male B6C3F1 mice 23 hours after intraperitoneal injection of furan doses of up to 350 mg/kg body weight or 42 hours after the administration of up to 100 mg/kg body weight (NTP 1993).

In a test with doses of up to 350 mg/kg body weight, furan did not induce chromosomal aberrations in the bone marrow of mice at the time of preparation after 17 hours. Two tests were carried out at a later time of preparation of 36 hours; because of toxicity, exposure was only possible up to a maximum dose of 250 mg/kg body weight. In these tests, the frequency of chromosomal aberrations was significantly increased at the highest tested dose of 250 mg/kg body weight (NTP 1993). This test provides evidence of a clastogenic potential of furan.

Reynolds et al. (1987) investigated mutations in activated oncogenes of furan-induced and spontaneous liver tumours in B6C3F1 mice to clarify the mechanism of tumour induction by furan. The spectrum of mutations in activated H-*ras* genes and the patterns of *ras* gene activation differed significantly between induced and spontaneous liver tumours. Mutations at codon 61 of the H-*ras* gene were observed in all spontaneous liver tumours. However, 60% of the oncogenes activated in induced liver tumours had mutations at a different codon of H-*ras* (codon 117) or mutations in different oncogenes (K-*ras*, *raf* and non-*ras*). G → T and G → C transversions were found in activated H-*ras* in liver adenomas and carcinomas that developed after exposure to furan; they were observed at codon 117 only in treated animals. The authors interpreted these new mutations in the *ras* genes of furan-induced liver tumours as a direct genotoxic effect of furan.

This interpretation is supported by an in vitro binding study with the furan metabolite cis-2-butene-1,4-dial in isolated deoxyribonucleotides. It showed that cis-2-

butene-1,4-dial formed diastereomeric adducts with 2′-deoxycytidine, 2′-deoxyguanosine and 2′-deoxyadenosine, but not with 2′-deoxythymidine. The metabolite binds to exocyclic and endocyclic nitrogen atoms of deoxyribonucleotides (Byrns et al. 2002). It has thus been demonstrated that this genotoxic metabolite of furan plays an important role in carcinogenicity.

The overall data suggest furan has genotoxic potential resulting from the metabolite cis-2-butene-1,4-dial.

5.7 Carcinogenicity

In a 2-year study carried out by the NTP with B6C3F1 mice (0, 8 and 15 mg/kg body weight and day) and Fischer 344/N rats (0, 2, 4 and 8 mg/kg body weight and day), animals died before the end of the study after gavage doses of furan (purity: > 99%) in corn oil. The incidences of hepatocellular adenomas and carcinomas were increased in a dose-dependent manner in both species (significantly in rats at the middle dose and above and in mice at the low dose and above), and a dose-dependent increase in leukaemia and a high incidence (86% to 100%) of intrahepatic bile duct carcinomas were observed in rats in all 3 dose groups. Mice were found to have adrenal phaeochromocytomas (a significant increase in both dose groups in the males and only in the high dose group in the females) (NTP 1993). The data are shown in Table 3.

The NTP study also included a stop-exposure study with 50 male rats that were given oral furan doses of 30 mg/kg body weight and day for 13 weeks and then observed up to the end of the 2 years (see Table 2). Groups of 10 animals were examined at the end of the 13-week exposure period or after 9, 15 or 24 months. Bile duct carcinomas were detected in 40/40 animals and hepatocellular carcinomas were found in 6/40 animals that survived at least 9 months. While bile duct carcinomas were detected in all 10 of the animals examined after 9 or 15 months, hepatocellular carcinomas were first observed in 2/10 rats after 15 months (NTP 1993). At the end of the 2-year observation period, the findings were more or less the same as those established in the 2-year study.

Groups of 12 young male Fischer 344 rats were treated with furan doses of 30 mg/kg body weight and day for 13 weeks, and groups of 10 Fischer 344 rats were treated with 30 mg/kg body weight and day for 6, 9 or 12 weeks. All rats were sacrificed 16 months after the beginning of exposure and examined with particular attention to the distribution of tumours in the individual liver lobes. The tumours occurred mainly in the right and caudate liver lobes. Intestinal-type bile duct carcinomas were observed in 4/9 rats after 6-week exposure, in 6/8 animals after 9-week exposure, in 5/7 animals after 12-week exposure and in 9/10 animals after 13-week exposure; hepatocellular carcinomas were found in only 2/10 rats after 13-week exposure (Elmore and Sirica 1993).

In a 2-year study with female B6C3F1 mice and exposure to furan at 0, 0.5, 1, 2, 4 or 8 mg/kg body weight, tumours developed at 4 mg/kg body weight and day and above (Goldsworthy and Foley 2001). As this study has not yet been published, there are no further data available and this study cannot be included in the evaluation.

Table 2 Liver findings in the stop-exposure study with male rats in the interim examinations

Author:	NTP (1993)
Substance:	furan (purity: 99%)
Species:	rat, F344/N, 50 ♂; groups of 10 animals examined after 13 weeks, 9 or 15 months
Exposure:	oral, gavage in corn oil
Dose:	rats: 0 or 30 mg furan/kg body weight and day
Duration:	exposure period: 13 weeks, 5 days/week observation period: up to the end of 2 years
Tumours:	

exposed rats	**examination after**		
	13 weeks	**9 months**	**15 months**
neoplasms:			
bile duct carcinomas	0/10 (0%)	10/10 (100%)	10/10 (100%)
hepatocellular carcinomas	0/10 (0%)	0/10 (0%)	2/10 (20%)
non-neoplastic lesions:			
biliary tract			
fibrosis	10/10 (100%)	10/10 (100%)	10/10 (100%)
hyperplasia	10/10 (100%)	10/10 (100%)	10/10 (100%)
chronic inflammation	0/10 (0%)	10/10 (100%)	10/10 (100%)
cysts	0/10 (0%)	10/10 (100%)	10/10 (100%)
hepatocytes			
cytomegaly	10/10 (100%)	10/10 (100%)	10/10 (100%)
degeneration	10/10 (100%)	10/10 (100%)	10/10 (100%)
hyperplasia	10/10 (100%)	10/10 (100%)	10/10 (100%)
necrosis	10/10 (100%)	10/10 (100%)	10/10 (100%)
vacuolation	10/10 (100%)	10/10 (100%)	10/10 (100%)
Kupffer's cells			
pigmentation	10/10 (100%)	10/10 (100%)	10/10 (100%)

Table 3 Carcinogenicity studies of furan

Author:	NTP (1993)				
Substance:	furan (purity: 99%)				
Species:	rat, F344/N, 50 ♂/50 ♀ mouse, B6C3F1, 50 ♂/50 ♀				
Exposure:	oral, gavage in corn oil				
Dose:	rats: 0, 2, 4 or 8 mg furan/kg body weight and day mice: 0, 8 or 15 mg furan/kg body weight and day				
Duration:	2 years, 5 days/week				
Toxicity:	2 mg/kg body weight and day and above: hepatotoxicity (see Section 5.2.2)				
Tumours:					
rats		**dose (mg/kg body weight and day)**			
		0	**2**	**4**	**8**
survival	♂	33/50 (66%)	28/50 (56%)	26/50 (52%)	16/50 (32%)
	♀	34/50 (68%)	32/50 (64%)	28/50 (56%)	19/50 (38%)
liver:					
bile duct carcinomas	♂	0/50 (0%)	43/50 (86%)***	48/50 (96%)***	49/50 (98%)***
	♀	0/50 (0%)	49/50 (98%)***	50/50 (100%)***	48/50 (96%)***
hepatocellular adenomas	♂	1/50 (2%)	4/50 (8%)	18/50 (36%)***	27/50 (54%)***
	♀	0/50 (0%)	2/50 (4%)	4/50 (8%)*	7/50 (14%)**
hepatocellular carcinomas	♂	0/50 (0%)	1/50 (2%)	6/50 (12%)**	18/50 (36%)***
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
hepatocellular adenomas or carcinomas	♂[a)]	1/50 (2%)	5/50 (10%)	22/50 (44%)***	35/50 (70%)***
	♀[b)]	0/50 (0%)	2/50 (4%)	4/50 (8%)*	8/50 (16%)***
mononuclear leukaemia	♂[c)]	8/50 (16%)	11/50 (22%)	17/50 (34%)*	25/50 (50%)***
	♀[d)]	8/50 (16%)	9/50 (18%)	17/50 (34%)*	21/50 (42%)**

Table 3 (continued)

mice		dose (mg/kg body weight and day)		
		0	**8**	**15**
survival	♂	33/50 (66%)	17/50 (56%)	16/50 (52%)
	♀	29/50 (58%)	25/50 (50%)	2/50 (4%)
liver:				
hepatocellular adenomas	♂	20/50 (40%)	33/50 (66%)***	42/50 (84%)***
	♀	5/50 (10%)	31/50 (62%)***	48/50 (96%)***
hepatocellular carcinomas	♂	7/50 (14%)	32/50 (64%)***	34/50 (68%)***
	♀	2/50 (4%)	7/50 (14%)	27/50 (54%)***
hepatocellular adenomas or carcinomas	♂[e]	26/50 (52%)	44/50 (88%)***	50/50 (100%)***
	♀[f]	7/50 (14%)	34/50 (68%)***	50/50 (100%)***
benign phaeochromocytomas in the adrenal medulla	♂[g]	1/49 (2%)	6/50 (12%)*	10/50 (20%)**
	♀[h]	2/50 (4%)	1/50 (2%)	6/50 (12%)*

[a] historical controls: 19/770 incidence, 2.5% ± 2.8% (mean ± standard deviation), range: 0%–10%
[b] historical controls: 9/770 incidence, 1.2% ± 2.7% (mean ± standard deviation), range: 0%–10%
[c] historical controls: 164/770 incidence, 21.3% ± 8.9% (mean ± standard deviation), range: 4%–38%
[d] historical controls: 206/770 incidence, 26.8% ± 7.0% (mean ± standard deviation), range: 16%–38%
[e] historical controls: 210/599 incidence, 35.1% ± 11.0% (mean ± standard deviation), range: 14%–52%
[f] historical controls: 60/597 incidence, 10.1% ± 4.3% (mean ± standard deviation), range: 2%–16%
[g] historical controls: 16/582 incidence, 2.7% ± 1.6% (mean ± standard deviation), range: 0%–4%
[h] historical controls: 9/584 incidence, 1.5% ± 2.4% (mean ± standard deviation), range: 0%–8%
***: p = 0.001 (trend test)
**: p = 0.01 (trend test)
*: p = 0.05 (trend test)
historical control data and significances from NTP (1993)

5.8 Other effects

Studies in freshly isolated rat hepatocytes after exposure in vitro (2 to 100 µM furan) or in vivo (0 to 30 mg furan/kg body weight) revealed an irreversible reduction in ATP that was dependent on the concentration and incubation time. Depletion of ATP occurred prior to cell death and could be prevented by a cytochrome P450 inhibitor (1-phenylimidazole). Various test runs showed that the furan metabolite 2-butene-1,4-dial uncouples the mitochondrial oxidative phosphorylation of hepatocytes in vitro and in vivo (Mugford et al. 1997).

Dose-dependent cytotoxicity and glutathione depletion were observed when isolated hepatocytes of male Fischer 344 rats were incubated with furan doses of 136 to 6808 µg/l for 4 hours and then cultivated in a monolayer culture for an additional 25 hours. Both were prevented by the addition of 1-phenylimidazole to the cultures. The furan concentrations corresponded to the tissue concentrations calculated for hepatotoxic effects after oral administration of furan (IARC 1995).

Immunohistochemical examinations in rat livers revealed a significantly increased number of CDX1-positive cells (intestine-specific transcription factor) in early intestinal metaplasia after oral administration of furan for 3 weeks. CDX1 was expressed only in mucin-producing glands of hepatic cholangiofibrotic tissue induced by furan (Ren et al. 2000). CDX1 genes are characteristic of intestinal cells (Bonner et al. 1995). A study in young male Fischer 344 rats showed that intestinal metaplasia, which develops at an early stage in the liver, and the later developing bile duct carcinomas expressed c-*neu* and c-*met* after treatment with furan for 6 weeks (no other details). However, the surrounding non-neoplastic tissue did not express c-*met* or c-*neu* (Radaeva et al. 1999).

A study of the gene expression of hepatotoxic substances with furan doses of 40 mg/kg body weight and day administered for 1, 3, 7 or 14 days showed that furan inhibited the expression of a series of metabolic genes. Furan induced genes which react to cellular and oxidative stress, and "multidrug resistance" proteins. Furan inhibited the expression of various organic ion transporters in the liver, for example the organic anion transporters 3, K1 and polypeptide 1, and the transport pump that removes the bile salts from the cells (Huang et al. 2004).

The treatment of male Fischer 344 rats with furan doses of 8 mg/kg body weight and day in corn oil for 1, 3 or 6 weeks resulted in a 35-fold increase in the percentage of hepatocytes in the S-phase compared with that in the control animals. Northern blot analysis of mRNA expression in the liver showed a doubling of H-*ras* expression in the oncogenes, but no measurable expression of *fos* in weeks 1, 3 or 6 of treatment; a 10-fold increase in the expression of *myc* (precursors of cell division) was observed at the end of week 6 (IARC 1995).

Cytochrome P450 levels in liver microsomes were decreased 24 hours after single oral furan doses of 8 and 25 mg/kg body weight compared with those in the control animals (levels decreased to 90% and 71%, respectively) (Parmar and Burka 1993). This result was substantiated by a study with furan doses of 40 mg/kg body weight and day administered to Sprague Dawley rats for 1, 3, 7 and 14 days. Here, furan inhibited the expression of genes that are, for example, involved in general metabolism and also in lipid metabolism (Huang et al. 2004).

6 Manifesto (MAK value/classification)

There are no appropriate data available for the assessment of the effects of furan in humans.

The data suggest that furan has genotoxic effects because the substance has clastogenic potential and the metabolite cis-2-butene-1,4-dial is mutagenic in the aldehyde-sensitive Salmonella strain TA104. In addition, cis-2-butene-1,4-dial forms adducts with deoxyribonucleotides of guanine, cytosine and adenine. Also, mutations that were not observed in spontaneous liver tumours were found in the activated oncogenes of furan-induced liver tumours.

In the available studies, furan had toxic effects in vivo mainly on the bile duct and liver and induced hyperplasia and tumours accompanied by compensatory cell proliferation after long-term administration. A 2-year study with rats and mice revealed a high dose-dependent incidence of adenomas and carcinomas in the livers of both species. Furthermore, phaeochromocytomas were observed in mice and bile duct carcinomas were found in rats. The incidences of bile duct carcinomas were already so high at the low dose of 2 mg/kg body weight and day that a dose–response relationship could not be established. In addition, damage to the haematopoietic system (bone marrow, lymph nodes and spleen) and a dose-dependent increase in mononuclear leukaemia were observed in rats (NTP 1993). On the basis of the available data it is not possible to derive a dose level at which no significant contribution to the cancer risk would be expected in rats and above all in humans. PBPK model calculations could only predict that humans have a lower body burden than rats and mice given the same external exposure (Kedderis and Held 1996). A possible limit value would have to be derived from the uncoupling effect or other factors that induce cell destruction. As mechanistic findings only relate to damage to the liver, they cannot explain the development of all the tumour types that occurred. It is currently unclear whether a non-hepatotoxic furan concentration may nevertheless cause tumours of the bile ducts, lesions in the haematopoietic system and leukaemia in rats. Nor is it known whether bile duct epithelial cells or liver stem cells are the starting cells of bile duct carcinomas. On the basis of the available data, furan is therefore classified in Carcinogen Category 2. It might be reclassified in Carcinogen Category 4 should experimental studies provide the evidence necessary to establish a MAK value and the role of genotoxicity have been clarified. These studies would have to be carried out in rats and mice to identify the different target organs in both species.

Based on the theoretical models of Fiserova-Bergerova et al. (1990) and Guy and Potts (1993), it must be assumed that furan is readily absorbed through the skin. Because of its very high vapour pressure, this theoretical ability to penetrate the skin will probably not be completely exhausted in practice. As furan was carcinogenic in animal studies and genotoxic effects cannot be ruled out, an additional carcinogenic risk has to be assumed for dermal exposure and the estimated amounts absorbed. Furan has therefore been designated with an “H” (for substances which can be absorbed through the skin in relevant amounts).

The sensitizing effects of furan cannot be evaluated because of a lack of data; therefore, furan has not been designated with “Sh” or “Sa” (for substances which can cause sensitization of the skin or airways).

In Europe, furan was classified in EU Category M3 as a possible germ cell mutagen. However, as the available studies only revealed damage to the ovaries or testes if the animals had been exposed to high doses and already had marked liver damage, furan has not been classified in any of the categories for germ cell mutagens.

7 References

Bitsch A, Klöhn P-C, Hadjiolov N, Bergmann O, Neumann H-G (1999) New insights into carcinogenesis of the classical model arylamine 2-acetylaminofluorene. Cancer Lett 143: 223–227

Bitsch A, Hadjiolov N, Klöhn P-C, Bergmann O, Zwirner-Baier I, Neumann H-G (2000) Dose response of early effects related to tumor promotion of 2-acetylaminofluorene. Toxicol Sci 55: 44–51

Bonner CA, Loftus SK, Wasmuth JJ (1995) Isolation, characterization, and precise physical localization of human CDX1, a caudal-type homeobox gene. Genomics 28: 206–211

Burka LT, Washburn KD, Irwin RD (1991) Disposition of [14C]furan in the male F344 rat. J Toxicol Environ Health 34: 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. Chem Res Toxicol 15: 373–379

Carfagna MA, Held SD, Kedderis GL (1993) Furan-induced cytolethality in isolated rat hepatocytes: correspondence with in vivo dosimetry. Toxicol Appl Pharmacol 123: 265–273

Chen L-J, Hecht SS, Peterson LA (1995) Identification of cis-2-butene-1,4-dial as a microsomal metabolite of furan. Chem Res Toxicol 8: 903–906

Chen L-J, Hecht SS, Peterson LA (1997) Characterization of amino acid and glutathione adducts of cis-2-butene-1,4-dial, a reactive metabolite of furan. Chem Res Toxicol 10: 866–874

Egle JL, Gochberg BJ (1979) Respiratory retention and acute toxicity of furan. Am Ind Hyg Assoc J 40: 310–314

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 Res 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 Res 53: 254–259

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17: 617–635

Fransson-Steen R, Goldsworthy TL, Kedderis GL, Maronpot RR (1997) Furan-induced liver cell proliferation and apoptosis in female B6C3F1 mice. Toxicology 118: 195–204

Goldsworthy T, Foley J (2001) Dose response relationships between furan-induced cytotoxicity and liver cancer (abstract). Toxicol Pathol 29: 710

Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. Am J Ind Med 23: 711–719

Huang Q, Jin X, Gaillard ET, Knight BL, Pack FD, Stoltz JH, Jayadev S, Blanchard KT (2004) Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants. Mutat Res 549: 147–168

IARC (International Agency for Research of Cancer) (1995) Furan. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol 63, IARC, Lyon, FR, 393–407

Kedderis GL, Carfagna MA, Held SD, Batra R, Murphy JE, Gargas ML (1993) Kinetic analysis of furan biotransformation by F-344 rats in vivo and in vitro. Toxicol Appl Pharmacol 123: 274–282

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

Kedderis GL, Ploch SA (1999) The biochemical toxicology of furan. Chemical Industry Institute of Toxicology (CIIT) Activities 19: 1–6

Keller BJ, Marsman DS, Popp JA, Thurman RG (1992) Several nongenotoxic carcinogens uncouple mitochondrial oxidative phosphorylation. Biochim Biophys Acta 1102: 237–244

Klöhn P-C, Soriano ME, Irwin W, Penzo D, Scorrano L, Bitsch A, Neumann H-G, Bernardi P (2003) Early resistance of cell death and to onset of the mitochondrial permeability transition during hepatocarcinogenesis with 2-acetylaminofluorene. Proc Natl Acad Sci USA 100: 10014–10019

Masuda Y, Nakayama N, Yamaguchi A, Murohashi M (1984) The effects of diethyldithiocarbamate and carbon disulfide on acute nephrotoxicity induced by furan, bromobenzene and cephaloridine in mice. Jpn J Pharmacol 34: 221–229

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ (1988) Responses of the L5178Y tk+/tk–-mouse lymphoma cell forward mutation assay: III 72 coded chemicals. Environ Mol Mutagen 12: 85–154

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E (1986) Salmonella mutagenicity tests: II results from the testing of 270 chemicals. Environ Mutagen 8, Suppl 7: 1–119

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

Nicotera P, Bellomo G, Orrenius S (1990) The role of Ca2+ in cell killing. Chem Res Toxicol 3: 484–494

NIOSH (National Institute for Occupational Safety and Health) (2002) Furan, Registry of toxic effects of chemical substances (RTECS), excerpt from databank, NIOSH, Cincinnati, OH, USA

NTP (National Toxicology Program) (1993) Toxicology and carcinogenesis studies of furan (CAS No. 110-00-9) in F344/N rats and B6C3F1 mice (Gavage Studies). NTP Technical Report Series No. 402, US Department of Health and Human Services, National Institutes of Health, Bethesda, MD, USA

Parmar D, Burka LT (1993) Studies on the interaction of furan with hepatic cytochrome P-450. J Biochem Toxicol 8: 1–9

Peterson LA, Naruko KC, Predecki DP (2000) A reactive metabolite of furan, cis-2-butene-1,4-dial, is mutagenic in the ames assay. Chem Res Toxicol 13: 531–534

Radaeva S, Ferreira-Gonzalez A, Sirica AE (1999) Overexpression of C-NEU and C-MET during rat liver cholangiocarcinogenesis: a link between biliary intestinal metaplasia and mucin-producing cholangiocarcinoma. Hepatology 29: 1453–1462

Ren P, Silberg DG, Sirica AE (2000) Expression of an intestine-specific transcription factor (CDX1) in intestinal metaplasia and in subsequently developed intestinal type of cholangiocarcinoma in rat liver. Am J Pathol 156: 621–627

Reynolds SH, Stowers SJ, Patterson RM, Maronpot RR, Aaronson SA, Anderson MW (1987) Activated oncogenes in B6C3F1 mouse liver tumors: implications for risk assessment. Science 237: 1309–1316

Römpp (1999) Kompakt, Basislexikon Chemie (Compact, basic lexicon on chemistry) (German), Thieme Verlag, Stuttgart New York

Sell S (2002) Cellular origin of hepatocellular carcinomas. Semin Cell Dev Biol 13: 419–424

Sirica AE, Gainey TW, Mumaw VR (1994) Ductular Hepatocytes – Evidence for a bile ductular cell origin in furan-treated rats. Am J Pathol 145: 375–383

SRC (Syracuse Research Corporation) (2005)
https://www.srcinc.com/what-we-do/environmental/scientific-databases.html
http://www.syrres.com/esc/physdemo.htm

Stich HF, Rosin MP, Wu CH, Powrie WD (1981) Clastogenicity of furans found in food. Cancer Lett 13: 89–95

Wilson DM, Goldsworthy TL, Popp JA, Butterworth BE (1992) Evaluation of genotoxicity, pathological lesions, and cell proliferation in livers of rats and mice treated with furan. Environ Mol Mutagen 19: 209–222

completed 17.02.2005