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Working Group on Flavourings

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Opinion
of the Scientific Committee on Food
on furfural and furfural diethylacetal

(expressed on 2 December 2002)

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Terms of Reference

The Committee is asked to advise the Commission on substances used as flavouring substances or present in flavourings or present in other food ingredients with flavouring properties for which existing toxicological data indicate that restrictions of use or presence might be necessary to ensure safety for human health.

In particular the Committee is asked to advise the Commission on the implications for human health of the presence of furfural in the diet.

Introduction

Beside furfural, furfural diethylacetal is listed in the register of chemically defined flavouring substances laid down in Commission Decision 1999/217/EC (EC, 1999), as last amended by Commission Decision 2002/113/EC (EC, 2002). The Committee was aware that diethylacetals are readily hydrolysed to the corresponding aldehyde and ethanol under acid conditions such as occur in the human stomach. Since furfural diethylacetal would undergo such hydrolysis to furfural, the conclusions of this opinion are also valid for furfural diethylacetal.

Previous evaluations

In an earlier evaluation by the Scientific Committee on Food (SCF, 1995), furfural was placed in category 4 (unsuitable for use due to evidence of toxicity).

The Committee of Experts on Flavouring Substances of the Council of Europe evaluated furfural as follows (CEFS, 1998): "Many short term and long term studies have shown the hepatoxicity of furfural. Carcinogenic effects occurred in male rats and male mice at the highest dosage (60 mg/kg in rat; 175 mg/kg in mice)."…"Furfural should be considered as a non-genotoxic compound and the highest hepatoxic dose levels administered to male rat or male mice could lead to cell proliferation and cell death and, after prolonged exposure, to liver tumours. If the Committee considers furfural as a non-genotoxic compound, a NOEL can be set at 30 mg/kg bw. Using a safety factor of 100, a TDI of 300 µg/kg/day is proposed."

In the 45th CEFS meeting a majority decision was taken to delete furfural from the list of active principles and consider it as a special case because it occurs only in trace amounts in natural sources of flavourings. In addition, it was deleted from the 4th edition of the Blue Book (Vol. 1) (CEFS, 1999).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was unable to allocate an ADI to furfural at its thirty-ninth meeting (JECFA, 1992) and its fifty-first meeting (JECFA, 1999) due to concern about tumours observed in male mice given furfural and the fact that no NOEL was established for hepatotoxicity in rats.
At its Fifty-fifth meeting (JECFA, 2000), the JECFA concluded that new data from a rat toxicity study enabled a NOEL to be determined and a UDS study by Edwards (1999) was conducted in response to a request by the JECFA for a study on the formation of DNA adducts in order to clarify whether the tumours seen in the National Toxicology Program (NTP) study in mice arose from a genotoxic mechanism or were secondary to the increased incidence of liver necrosis. Although the UDS assay was not a direct assay for adduct formation the JECFA considered that its requirements for additional studies to establish a NOEL and clarify the potential genotoxicity had been met. It concluded that the evidence provided indicated a non-genotoxic mechanism for tumour formation in the mouse liver and hence an ADI could be established. The hydrolysis of furfuryl esters and interconversion of furfuryl alcohol and furfural, with subsequent oxidation to furoic acid provided the basis for the JECFA allocating a Group ADI of 0-0.5 mg/kg bw for furfural, furfuryl alcohol, furfuryl acetate, furfuryl propionate, furfuryl pentanoate, furfuryl octanoate, furfuryl 3-methylbutanoate, methyl 2-furoate, propyl 2-furoate, amyl 2-furoate, hexyl 2-furoate and octyl 2-furoate.

Current Regulatory Status

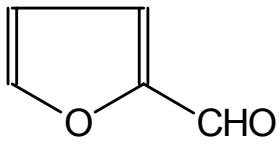
Furfural and furfural diethylacetal are listed in the register of chemically defined flavouring substances (EC, 2002).

Furfural has Flavour and Essence Manufacturers Association (FEMA) GRAS status in the United States.

Chemical characterisation

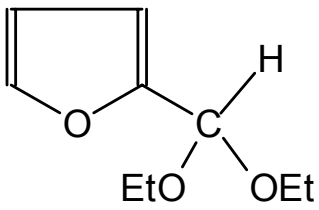
Name:	Furfural
Synonyms:	Furfuraldehyde; 2-Furancarboxaldehyde; Fural; 2-Formylfuran; 2-Furaldehyde; Pyromucic aldehyde; 2-Furylcarboxaldehyde
FL No:	13.018
CAS No:	98-01-1
FEMA No:	2489
CoE No:	2014
EINECS:	202-627-7

Structure:



Name:	Furfural diethylacetal
Synonyms:	2-furaldehyde diethylacetal
FL No:	13.126
CAS No:	13529-27-6
FEMA No:	-
CoE No.	-
EINECS:	236-872-6

Structure:



Exposure assessment

Furfural, together with the corresponding furfuryl alcohol, occurs naturally in many fruits and in tea, coffee and cocoa. It is produced during the acid hydrolysis or heating of polysaccharides containing hexose or pentose fragments (Maarse *et al.*, 1994) and highest concentrations are found in cocoa and coffee (55-255 mg/kg), alcoholic beverages (1-33 mg/kg) and whole grain bread (up to 26 mg/kg). The estimated daily per capita intake from use as a flavouring agent in Europe (eaters only) is approx. 10 µg/kg bw/day (IOFI, 1995). The intake from natural sources has been estimated to be about 300 µg/kg bw/day in the United States of America (Stofberg & Grundschober, 1987).
Furfural is used as a flavouring substance ingredient in many food categories. The average use levels according to Fenaroli (1995) are in the range from 4 mg/kg in gravies to 63 mg/kg in

meat products. The theoretical maximum daily intake (assumption: consumers consume at all times all flavoured foods with maximum concentrations) has been estimated to be 136 µg/kg bw (CEFS, 1998).

Hazard identification/characterisation

Absorption, metabolism and excretion

Furfural is converted to furfuryl alcohol by enteric bacteria, which can also be formed by hydrolysis of esters of furfuryl alcohol (Boopathy *et al*., 1993, Grundschober, 1977; Buck, 2000). Furfural and the corresponding alcohol are rapidly absorbed from the g.i. tract at doses of 0.1 to 200 mg/kg bw and virtually totally excreted mainly in urine within 24 hours (Nomeir *et al.,* 1992).
After absorption, furfuryl alcohol is oxidised to furfural and further to 2-furoic acid. Furoic acid is then conjugated with glycine or converted to 2-furanacryloyl-CoA and then to 2-furanacrylic acid and its glycine conjugate (Parkash & Caldwell, 1994). Some conversion to CO_2 occurs in rodents (presumably from decarboxylation of furoic acid) but not in humans.

In rodents, the decarboxylation may be preceded by epoxidation or hydroxylation of the furan ring yielding reactive intermediates (e.g. furfural-2,3-epoxide, acetyl acrolein or *alpha*-ketoglutaric acid) which may be decarboxylated or conjugated with glutathione (Ramsdell & Eaton, 1990; Koenig & Andreesen, 1990; Gupta *et al*., 1991; Mishra *et al.,* 1991). These latter pathways appear to be insignificant in humans at low levels of exposure (Flek & Sedivec, 1978).

Acute toxicity

The oral LD_{50} for furfural was reported to be 127 mg/kg bw in rats (Jenner *et al.,* 1964) and 333 mg/kg bw in mice (Boyland *et al.,* 1940).

Sub-acute/sub-chronic toxicity

Groups of five male and five female Fischer rats that received furfural by gavage at doses of 0, 15, 30, 60, 120 or 240 mg/kg bw for a total of 12 doses over 16 days did not display any gross or histopathological abnormalities at termination although eight of ten rats at the top dose were reported to have died due to gavaging accidents. In a study using a similar protocol, groups of five male and five female B6C3F1 mice were given 12 doses of furfural by gavage at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw. No gross or histopathological abnormalities were reported but one male of the top dose group and two females were stated to have died from gavaging accidents.

In a 13 week study, groups of 10 male and 10 female mice were given furfural by gavage in corn oil at doses of 0, 75, 150, 300, 600 or 1200 mg/kg bw per day on five days per week. All the mice in the top dose group and all but one of each sex in the 600 mg/kg bw groups died before the end of the study. Females at 75-300 mg/kg bw and males at 300 mg/kg bw per day showed increased liver weight. Most males in the two highest dose groups and one in each of the 300 and 150 mg/kg bw dose groups showed centrilobular coagulative necrosis of hepatocytes; only two females in the highest group showed similar lesions. The lesion was accompanied by inflammation with mild mononuclear inflammatory cell infiltrate (NTP, 1990).

In a similar 13-week study in rats, furfural was administered at doses of 0, 11, 22, 45, 90 or 180 mg/kg bw per day by gavage on 5 days per week to groups of 10 males and 10 females. Only one male in the top dose group survived to termination. The liver and kidney weights of males in the two highest dose groups were increased and there was an increase in cytoplasmic vacuolation in hepatocytes in all groups of treated males. No lesions were reported in the kidneys (NTP, 1990).

In a study of unusual design, rats treated with furfural for 90 days at doses varying from 20 mg/kg diet to 40 mg/kg diet showed marked cholangiofibrosis with fibrous widening of Glisson's sheath, bile duct proliferation and parenchymal damage consisting of necrosis and hydropic degeneration of hepatocytes. However, furfural did not cause hepatocellular hyperplastic changes (Shimizu & Kanisawa, 1986; Shimizu *et al.,1989*).

A study was conducted to examine the mode of action by which furfural was hepatotoxic. Rats were dosed with furfural in the diet at approx. 1200 mg/kg bw for 30 days and 1700 mg/kg bw thereafter. The furfural was removed after 15, 30, 60, 90, 120 or 150 days and the animals killed 14 days later. Increased duration of exposure was associated with increased numbers and size of foci positive for placental type glutathione-S-transferase (Pickett & Lu, 1988).

In response to a JECFA requirement for the establishment of a NOEL for hepatotoxicity, a 14 day pilot study and a subsequent 90-day study were conducted in F344/N rats in which the animals were given microencapsulated furfural in the diet to provide target dose levels of 0,30, 60, 90, 120 and 180 mg/kg bw per day. In the pilot study a NOEL of 120 mg/kg bw/day was established. At the higher dose, there was an increase in liver weight (Jonker, 2000a). In the 90 day study, 10 animals of each sex were used at each dose level. There were no clinical signs of toxicity in any treatment group and food consumption and body weight gain were unaffected. Some haematological changes (decrease in erythrocytes in males in the highest dose group with increased cell volume and mean corpuscular haemoglobin in the top two dose groups males were observed. In females, there was a decrease in serum alkaline phosphatase, an increase in *gamma*-glutamyltransferase and an increase in plasma albumin in the highest dose group. In males in the high dose group there was a decrease in ALAT, an increase in plasma albumin and albumin/globulin ratio. At necropsy, an increase in liver weight was

observed in the top dose group only with no gross pathological changes. Slight histopathological changes occurred in liver of males of the 90 and 180 mg/kg bw dose group in the perilobular region characterised by cells with less coarse cytoplasm with eosinophilic clumps and a less dense periphery and more prominent nucleoli. No such effects were seen in females and there was no sign of hepatotoxic effects such as degeneration, necrosis or inflammation, nor of bile duct proliferation. The NOEL established from this study was 54 mg/kg bw/day (Jonker, 2000b).

Long-term studies of chronic toxicity and carcinogenicity

In a two-year study in B6C3F1 mice, 50 animals of each sex received doses of 0, 50, 100 or 175 mg/kg bw by gavage in corn oil on 5 days per week. At termination, there was no significant effect of furfural on body weight or survival. Histological examination showed chronic inflammation, necrosis and pigmentation in the liver of males in the two highest dose groups and in females of the top dose group only. Hepatocellular adenomas and carcinomas were observed in all dose groups, including controls but these tumours occurred with significantly increased incidence only in males of the top dose group. The incidence of carcinomas was similar in high dose females and controls. Tumours of other organs occurred only with low incidence and with no dose response relationship. Slight increases in the incidence of hyperplasia and papillomas in the forestomach of female mice were considered by the authors to be due to the irritating effect of gavage administration and not of toxicological significance; none of the animals had malignant lesions of the forestomach (NTP, 1990).

In a similar study in Fischer 344/N rats, furfural was administered at doses of 0, 30 or 60 mg/kg bw by gavage in corn oil to 50 animals of each sex. Mild centrilobular hepatocellular necrosis occurred in all groups but the incidence did not appear to be dose related, particularly in females where the incidence was inversely related to dose. Bile duct hyperplasia occurred with high incidence in all groups, including controls and did not appear to be treatment related. Focal bile duct dysplasia was seen in one male at the intermediate dose level and bile duct hyperplasia accompanied by fibrosis occurred in two males in the top dose group. One control male had a hepatocellular adenoma and two males in the high dose group had a cholangiocarcinoma (a rare tumour in historical controls). It was considered by the authors that although the incidence of this lesion was not statistically significant it offered some evidence of carcinogenicity.

Special studies on genotoxicity

Negative or weakly positive results have been obtained for most bacterial tests for genotoxicity. In particular, positive results were obtained in three out of several assays for reverse mutation in *Salmonella typhimurium* at relatively high concentrations in the absence of metabolic activation. Furfural was found to be clearly genotoxic in cultured mammalian cells at the gene and chromosome level in the absence of metabolic activation. It induced SCE

in cultured CHO cells and human lymphocytes. It was gentoxic in Drosophila in somatic cells (Wing spot test by inhalation) and germ cells (sex-chromosome loss by injection). It did not induce reciprocal translocations and sex-linked recessive lethal mutations, with only a doubtful increase in one study in Drosophila. Furfural was not genotoxic in any *in vivo* mammalian assays for chromosome aberrations, SCE or UDS. The results of genotoxicity studies on furfural are summarised in Table 1.

Table 1: Special studies on genotoxicity

End-Point	Test object	Concentration	Result	Reference
In vitro				
Reverse mutation	*S.typhimurium* TA100, TA98, TA1535	0.05-60 µmol/plate	Weakly positive (TA100)[b]	Loquet *et al.,* 1981
	S.typhimurium TA100, TA98, TA102	≤1.2 mmol/plate	Negative[a]	Aeschbacher *et al.,*1989
	S.typhimurium TA100, TA98	0.165-0.660 µmol/plate	Negative[a]	Shinohara *et al.,* 1986
	S.typhimurium TA102, TA104	5-500 µg/plate	Positive (TA104)	Shane *et al.,*1988
	S.typhimurium TA98, TA100,TA1535, TA1537	33.3-6666 µmol/plate	Negative[a]	Mortelmans *et al.,*1986
	S.typhimurium TA98, TA100	1-15 µL/plate	Positive[a] (TA100)	Zdzienicka *et al.,*1978
	S.typhimurium TA98, TA100	7 µL/plate	Negative[a]	Sasaki & Endo, 1978
	S.typhimurium TA100	4.44 µmol/plate	Negative[a]	Osawa & Namiki, 1982
	S.typhimurium TA98, TA100, TA104 *E.coli*WP2uvrA/PKM101	20 µL/plate	Negative[a]	McMahon *et al.,*1979
	S.typhimurium TA104	1 µmol (max. non-toxic dose)	Negative[b]	Marnett *et al.*, 1985
Umu gene expression	*S.typhimurium* TA1535/pSK/002	1932 µg/mL	Negative[a]	Nakamura *et al., 1987*
Rec assay	*B.subtilis* H17, M45	1.7-17 mg/disk	Positive[a]	Shinohara *et al.,* 1986
	B.subtilis H17, M45	1 mg/disk	Negative[a]	Matsui *et al.,* 1989
Forward mutation	L5178Y*tk*$^{+/-}$ *mouse lymphoma cells*	25-800 µg/mL	Positive[b]	McGregor *et al.,*1988
Chromosomal aberration	Chinese hamster ovary cells	10-40 mM	Positive[a]	Stich, 1981a, 1981b
	Chinese hamster ovary cells	200-1230 µg/mL	Positive[a]	Galloway *et al*., 1985
	Chinese hamster ovary cells	1.5-5000 µg/mL	Positive[a]	Gudi & Schadly., 1996
	Chinese hamster V79 cells	500-2000 µg/mL	Positive[a]	Nishi *et al*., 1989
Sister chromatid exchange	Chinese hamster ovary cells	11.7-3890 µg/mL	Positive[a]	Galloway *et al*., 1985
	Human peripheral lymphocytes	3.5-14x10^{-5} M	Positive[b]	Gomez-Arroyo & Souza, 1985

Unscheduled DNA synthesis	Human liver slices	0.14 mmol/L 0-25 mmol/L	Negative	Lake *et al.,* 1998
In vivo				
Sex-linked recessive lethal test	*D.melanogaster* *D.melanogaster*	1000 ppm, in diet 100 ppm, by injection	Negative Positive	Woodruff *et al*. 1985
Wing spot test	*D.melanogaster*	3750-7500 ppm by aerial exposure	Positive	Rodriguez-Arnaiz *et al.,* 1989
, Sex-chromosome loss	*D.melanogaster*	3750-5000 ppm, in diet and by injection	Positive on injection	Rodriguez-Arnaiz *et al.,* 1989, 1992
Reciprocal translocation	*D.melanogaster*	1000 ppm, in diet	Negative	Woodruff *et al*. 1985
Sister chromatid exchange/ chromosomal aberration	$B6C3F_1$ mouse bone marrow cells	50-200 mg/kg bw, once i.p.	Negative	National Toxicology Program 1990
Somatic chromosomal aberration	Swiss albino mouse bone marrow cells	4000 ppm for 5 days, in diet	Negative	Subramanyam *et al*., 1989[+]
Sperm head abnormalities	Swiss albino mouse	4000 ppm for 5 weeks, in diet	Negative	Subramanyam *et al*., 1989[+]
Unscheduled DNA synthesis	Fischer 344 rat hepatocytes	5.0, 16.7 or 50 mg/kg bw, orally	Negative	Phillips.*et al*., 1997
	$B6C3F_1$ mouse hepatocytes	50, 175 or 320 mg/kg bw, orally	Negative	Edwards, 1999

[a] with and without metabolic activation
[b] without metabolic activation
[c] with metabolic activation
[+]abstract only; no details available

Reproductive and Developmental toxicity

There are few data on the reproductive toxicity of furfural. In one limited study, five groups of eight pregnant rats received furfural by gavage in water at doses of 10, 50, 100, 500 or 1000 mg/kg bw/day on days 6-15 of gestation. Excessive deaths occurred at the two top doses and further groups were given 150, 250 and 350 mg/kg bw per day. Because of excessive mortality, all animals receiving doses of 250 mg/kg bw and higher were killed after 6 days. All other females survived except one that died within 1 hour of dosing, probably as a result of a dosing accident. Intrauterine growth and survival were not affected at doses of 10 – 150 mg/kg bw and no developmental variations were noted (US EPA, 1997).

Summary of the hazard identification/characterisation

Furfural is rapidly absorbed and metabolised by oxidation of the aldehydic function to furoic acid, most of which is conjugated with glycine and excreted. A small amount is condensed with CoA to form furanacroylCoA. The resultant furanacryloic acid is conjugated with glycine and excreted in urine. Since furfuryl alcohol is oxidised *in vivo* to furfural and then follows the same metabolic pathway as furfural, the alcohol, and its esters that are hydrolysed by intestinal enzymes may be considered together with furfural.

Furfural is clearly hepatotoxic in short-term studies at daily doses of 90 mg/kg bw and above in male rats and 150 mg/kg bw and above in mice. The toxicity may have been exaggerated by the vehicle used in gavage studies (corn oil) as a dose of 150 mg/kg bw was the highest dose tolerated in the rat in the developmental study where the vehicle was water.

Furfural was essentially negative in Salmonella reverse mutation assays and other bacterial assays but was directly genotoxic in cultured mammalian cells at the gene and chromosome levels. Furfural was genotoxic in Drosophila; it was negative *in vivo* in mammalian assays at the chromosome level and in a UDS assay.

In long-term toxicity/carcinogenicity studies, the combined incidence of hepatocellular adenomas and carcinomas was increased at the highest dose of 175mg/kg bw in male mice but not females. Hepatotoxicity (focal and multifocal necrosis and chronic inflammation) occurred in all groups including controls but was markedly more frequent in the high dose mice.

Long-term studies in rats led to bile duct hyperplasia and cholangiofibrosis in all animals (controls had the highest incidence). Mild hepatocellular necrosis was seen in all groups, but at higher rates in males at the high dose.

The recent short-term study in rats was conducted in order to establish a NOEL for the hepatotoxicity, which was determined to be 54 mg/kg bw.

Conclusion

The Committee was of the opinion that the data were not totally convincing in demonstrating that the carcinogenicity of furfural was mediated via a thresholded mechanism and hence was unable to allocate an ADI to furfural at the present time. It was aware that a study in transgenic mice of the potential of furfural to induce gene mutations *in vivo* was in progress. The results were expected to be available in the near future and the Committee would wish to re-evaluate furfural in the light of the results of this study.

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