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Flavour and Extract Manufacturers Association, 1620 I Street, NW. Suite 925, Washington, DC 20006, USA and Research Institute for Fragrance Materials, Hackensack, New Jersey, USA

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The FEMA GRAS Assessment of Furfural Used as a Flavour Ingredient

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Flavor and Extract Manufacturers' Association, 1620 I Street, NW, Suite 925, Washington, DC 20006, USA and †Research Institute for Fragrance Materials, Hackensack, New Jersey, USA

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Summary—The Expert Panel of the Flavor and Extract Manufacturers' Association (FEMA) has assessed the safety of furfural for its continued use as a flavour ingredient. The safety assessment takes into account the current scientific information on exposure, metabolism, pharmacokinetics, toxicology, carcinogenicity and genotoxicity. Furfural was reaffirmed as GRAS (GRASr) as a flavour ingredient under conditions of intended use based on: (1) its mode of metabolic detoxication in humans; (2) its low level of flavour use compared with higher intake levels as a naturally occurring component of food; (3) the safety factor calculated from results of subchronic and chronic studies, (4) the lack of reactivity with DNA; and (5) the conclusion that the only statistically significant finding in the 2-year NTP bioassays, an increased incidence of hepatocellular adenomas and carcinomas in the high-dose group of male mice, was secondary to pronounced hepatotoxicity. Taken together, these data do not indicate any risk to human health under conditions of use as a flavour ingredient. This evidence of safety is supported by the occurrence of furfural as a natural component of traditional foods, at concentrations in the diet resulting in a 'natural intake' that is at least 100 times higher than the intake of furfural from use as a flavour ingredient. © *1997 Elsevier Science Ltd*

Abbreviations: ABS = chromosome aberrations; ADI = acceptable daily intake; BaP = benzo[*a*]pyrene; CHO = Chinese hamster ovary; CoA = coenzyme A; CYP450 = cytochrome *P*-450; DNA = deoxyribonucleic acid; DSB = double strand breaks; FAO/WHO = Food and Agriculture Organisation/World Health Organization; FEMA = The Flavor and Extract Manufacturers' Association; GRAS = generally recognized as safe; GRASa = GRAS affirmed; GRASr = GRAS reaffirmed; GST = glutathione *S*-transferase; IARC = International Agency for Research on Cancer; JECFA = Joint Expert Committee on Food Additives; NAS = National Academy of Sciences; NTP = National Toxicology Program; LD_{50} = median lethal dose; MLA = mouse lymphoma assay; ppm = parts per million; SAL = *Salmonella typhimurium*; SCE = sister chromatid exchanges; SLR = scientific literature review; UDS = unscheduled DNA synthesis.

EXPERT PANEL MEMBERS

John Doull, MD, PhD
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, Kansas

Jay I. Goodman, PhD
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, Michigan

Ian C. Munro, PhD
CanTox, Inc.
Mississauga, Ontario

Paul M. Newberne, DVM, PhD
Department of Pathology
Boston University
School of Medicine
Boston, Massachusetts

Philip S. Portoghese, PhD
Department of Medicinal Chemistry
University of Minnesota
Minneapolis, Minnesota

Robert L. Smith, PhD
Department of Biochemical and Experimental Pharmacology
St. Mary's Hospital
University of London
Paddington, London

Bernard M. Wagner, MD
New York University
School of Medicine
New York, New York

Bernard M. Wagner, Associates
Millburn, New Jersey

Carrol S. Weil, MA
Carrol S. Weil, Consultant
Pittsburgh, Pennsylvania

Lauren A. Woods, MD, PhD
Medical College of Virginia
Richmond, Virginia

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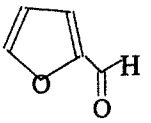
Furfural (FEMA No. 2489)



CAS No. 98-01-1

Synonyms: Fural
2-Furancarboxaldehyde
α-Furfuraldehyde
Furfuraldehyde
2-Furylcarboxaldehyde
Pyromucic aldehyde
2-Furaldehyde

Fig. 1. Structure of furfural.

I. Introduction

In 1960, the FEMA Expert Panel judged furfural to be "generally recognized as safe" (GRAS) under conditions of intended use as a flavouring substance in food (GRAS 3, Hall and Oser, 1965). In 1975, the Panel evaluated the available data and affirmed the GRAS status of furfural from use as a flavour ingredient (GRASa, unpublished). In 1993, furfural was evaluated at the thirty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1992). Based on the results of a 2-year carcinogenicity bioassay performed at the National Toxicology Program (NTP, 1990a) and positive results in selected *in vitro* and *in vivo* genotoxicity assays, the Committee considered that it could not allocate an Acceptable Daily Intake (ADI) to furfural. In 1995, the International Agency for Research on Cancer (IARC) evaluated these data and concluded that there is inadequate evidence in humans and limited evidence in animals for the carcinogenicity of furfural (IARC, 1995). In 1996, the FEMA Expert Panel performed a comprehensive review of all scientific data relevant to the safety evaluation of furfural from use as a flavour ingredient. This document contains their interpretation of the data. Additionally, it includes an interpretation of other data cited in previous safety assessments of furfural.

*The daily *per capita* intake ("eaters only") is a method adopted by the US Food and Drug Administration (FDA) to estimate "high" intake eaters. It is calculated as follows: (Annual volume, kg) × (1 × 10^6 mg/kg) × (1/26 × 10^6 people in the US) × (1/365 days) × (1/60 kg body weight) × (1/0.6) and is based on the assumptions that (1) only 10% of the population, the "eaters only" consumed the entire reported annual volume of a flavouring substance (NAS, 1987) and (2) only 60% of the flavour volume was reported by flavour manufacturers in the annual survey (NAS, 1987).

II. Exposure

A. Flavour use

Furfural is used as a flavour ingredient in 10 separate food categories (Hall and Oser, 1965) (Table 1). The average maximum use levels in these food categories are in the range from 4.3 parts per million (ppm) in gravies to 63 ppm in meat products.

The results of four US industry surveys performed over a 17-year period by the National Academy of Sciences (NAS) indicate that the annual volume of use of furfural as a flavour ingredient always has been less than 2500 kg (1640 kg, NAS, 1970; 2040 kg, NAS, 1975; 2300 kg, NAS, 1982; 594 kg, NAS, 1987). Based on the most recent reported annual volume of 594 kg (NAS, 1987), the estimated daily *per capita* intake ("eaters only") of furfural from use as a flavour ingredient is approximately 2 μg/kg*. The total estimated daily *per capita* intake ("eaters only") from use of furfural and furfuryl derivatives, which may form furfural *in vivo* (i.e. furfuryl alcohol and furfuryl esters), as flavour ingredients is less than 3 μg/kg* (NAS, 1987).

Loss of furfural associated with evaporation is significant for foods which are heated during processing. Therefore, the concentration of furfural consumed as a flavour ingredient in ready-to-eat products is often much lower than the concentration added as a flavour ingredient (Table 1). Furfural used as a flavour ingredient in foods that are not ready-to-eat will be significantly diluted during the food preparation or cooking process, prior to consumption.

B. Natural occurrence

Furfural and the corresponding furfuryl alcohol are virtually ubiquitous in nature. They are formed from the acid hydrolysis or heating of polysaccharides which contain pentose and hexose fragments (Fig. 1). Furfural has been detected in a broad

Table 1. Average maximum use levels of furfural

Food category	Average maximum levels (ppm)
Baked goods	50.0
Frozen dairy	44.3
Meat products	63.2
Soft candy	52.6
Gelatin puddings	32.8
Non-alcoholic beverages	28.4
Alcoholic beverages	7.0
Gravies	4.2
Hard candy	21.0
Chewing gum	56.4

range of fruits and fruit juices, wines, whiskeys, coffee and tea (CIVO–TNO, 1994). The highest concentrations of furfural in food have been reported in cocoa and coffee (55–255 ppm), alcoholic beverages (1–33 ppm) and wholegrain bread (26 ppm) (see Table 2). Furfuryl alcohol, which can be readily converted to furfural *in vivo* (Nomeir *et al.*, 1992; Rice, 1972), has been found in highest concentration in heated skim milk (230 ppm) and coffee (90–881 ppm). The total potential daily *per capita* intake of furfural and precursors of furfural (i.e. furfuryl alcohol and furfuryl esters) from consumption of foods in which they occur naturally (Stofberg and Grundschober, 1987) is approximately 0.3 mg/kg/day (i.e. approx. 300 μg/kg/day). Therefore, intake levels of furfural and furfuryl derivatives (less than 3 μg/kg) from use as flavour ingredients are approximately 100 times less than the levels consumed as natural components of traditional foods.

III. Absorption, distribution, metabolism and elimination

A. Aldehydes

Furfural contains a heteroaromatic furan ring with a reactive aldehyde functional group at the 2-position. The reactivity of the aldehyde function suggests that ingested furfural may not be absorbed intact. Low molecular weight aldehydes like furfural may undergo oxidation or condensation reactions associated with the aldehyde function either in digestive fluids prior to absorption, or in body fluids prior to entering the cell. For example, the reactivity of the aldehyde group is demonstrated by acetaldehyde and formaldehyde, both normal metabolic intermediates, which may produce toxic effects including the induction of tumours when administered under non-physiological conditions at high dose levels (Til *et al.*, 1989).

In addition, low molecular weight aldehydes have been reported to form imine adducts with soluble proteins, protein components of cell membranes, and thiol-containing molecules. Formation of adducts with albumin (Donahue *et al.*, 1983; Nagasawa *et al.*, 1980; Tuma *et al.*, 1984), haemoglobin (Peterson and Nguyen, 1985) and erythrocyte membranes (Gaines *et al.*, 1977) has been demonstrated *in vitro* and *in vivo*. Binding to membranes generally occurs with the protein component (Nomura and Lieber, 1981). Aldehydes also bind glutathione (Videlia *et al.*, 1982). Aldehyde dehydrogenase-catalysed oxidation of low molecular weight aldehydes requires glutathione (Eckfeldt and Yonetani, 1982), which suggests that the free aldehyde may be rapidly conjugated with glutathione *in vivo* to form a thiohemiacetal, which is subsequently oxidized to the corresponding acid (Brabec, 1993). Clearly, the reactivity of the aldehyde function will significantly curtail the intracellular concentration of free aldehyde.

Aldehyde that is present in the cell will be subject to rapid oxidation and conjugation of the resulting carboxylic acid. The competing extracellular and intracellular metabolic processes of low molecular weight aldehydes, particularly at low levels, are significant and must be considered when evaluating the fate of furfural. Therefore, toxicity, genotoxicity and carcinogenicity studies in which the various metabolic options (i.e. oxidation, conjugation, condensation) are not viable under study conditions cannot be used definitively to identify toxic, genotoxic or carcinogenic endpoints in animals.

B. Furfural

1. Laboratory animals

At doses in the range from 0.1 mg/kg to 200 mg/kg, furfural is rapidly absorbed from the gastrointestinal tract and distributed to the tissues, principally the liver and kidney. Tissue concentrations are generally proportional to the dose.

Furfural is metabolized primarily by oxidation of the aldehyde function in rats (Nomeir *et al.*, 1992; Parkash and Caldwell, 1994; Paul *et al.*, 1949; Rice, 1972) and mice (Parkash and Caldwell, 1994). Oxidation of furfural yields furoic acid which, as the CoA thioester, is either conjugated with glycine and excreted or condensed with acetyl CoA to form the chain-lengthened metabolite 2-furanacryloyl CoA (Fig. 2). 2-Furanacryloyl CoA conjugates with glycine and is excreted primarily in the urine. Additionally, in rats and mice, furoic acid decarboxylates to yield CO_2, presumably by oxidation of the furan ring. The absorption, tissue distribution, extent of metabolism, relative amounts of metabolites including CO_2, and rates of excretion in rodents were linear over the dose ranges investigated (0.1–200 mg/kg) (Nomeir *et al.*, 1992; Parkash and Caldwell, 1994) (Fig. 1).

Apparently, in animals, the condensation reaction of 2-furoyl CoA with acetyl CoA to yield furanacryloyl CoA is reversible, favouring formation of 2-furoyl CoA (Parkash and Caldwell, 1994). The observation that furoic acid is excreted in the urine of dogs given furanacrylic acid (Friedmann, 1911) supports this conclusion. Analogous reversible reactions between the CoA thioesters of benzoic acid and cinnamic acid *in vivo* favour benzoic acid (Nutley *et al.*, 1994). Excretion of unconjugated furoic acid and furanacrylic acid at higher dose levels in animals suggests that glycine conjugation in laboratory animals may be capacity-limited,

Table 2. Natural occurrence of furfural*

Food item	Concentration of furfural (ppm)
Apple (raw), apple juice, apricot (*Prunus armeniaca* L.), sweet cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.)	0.02–0.05
Orange juice (*Citrus sinensis* L. Osbeck)	trace
Orange peel oil, grapefruit juice (*C. paradisi*)	0.34
Bilberry (*Vaccinium myrtillus* L.)	0.02
American cranberry (*V. macrocarpon Ait.*)	0.1–0.3
Lingonberry (*V. vitis idaea* L.)	0.02
Blackcurrants—berries, guava (*Psidium guajava* L.)	0.0014–0.19
Grape (dried, sultana), peach (*Prunus persica* L.), pineapple (*Ananas comosus*), raspberry (*Rubus idaeus* L.), strawberry (*Fragaria* species), asparagus (raw), asparagus (cooked)	0.01
Carrot (*Daucus carota* L.), celery leaves (raw), onion (roasted), leek (heated), potato (raw), potato (cooked), bell pepper (*Capsicum annuum*)	0.005
Sauerkraut, tomato (*Lycopersicon esculentum* Mill.), cinnamon (*Cinnamomum zeylanicum Blume*), cloves (*Eugenia caryophyllata Thunberg*), *Mentha* species, wheaten bread	0.8–26
Crispbread, bread, other types, blue cheeses, parmesan, butter, yogurt, milk, chicken and turkey (raw), beef (boiled/cooked), beef (grilled/roasted)	0.02
Lamb and mutton, pork (heated), hop oil, beer	0–0.3
Cognac	0.6–33
Armagnac	2
Weinbrand	0.2–4.3
Grape brandy, other types, rum (all categories), rum (category I: total volatiles > 3600 ppm)	22
Rum (category II: total volatiles 1100–3600 ppm)	trace–25
Rum (category III: total volatiles 240–1100 ppm)	trace
Bourbon whisky	2–11.6
Irish whiskey	0.8–13.6
Malt whisky	10–37
Scotch blended whisky	1.1–30
Canadian whisky	0.3–0.8
Japanese whisky	0.5–4.5
Cider, sherry, white wine	trace–10.3
Red wine	0.005–0.05
Rose wine, port wine	2–34
Special wine, botrytized wine	0.13
Cocoa, coffee	55–255
Black tea	2–7
Green tea	0.1
Microbial fermented tea, tea (brewed)	0.3–0.8
Barley (roasted), filbert (roasted, *Corylus avellano*), peanut (roasted, *Arachis hypogea*), pecan (roasted), popcorn, potato chips (American)	0.08–0.2
Oat flakes (toasted), honey, soybean, arctic bramble (*Rubus arcticus* L., R. stell.), cloudberry (*Rubus chamaemorus* L.)	trace
Passion fruit juice (yellow), passion fruit (yellow), plum (raw), plum (salted and pickled)	2.58
Beans (*Phaseolus vulgaris* L.), mushroom (raw)	0.05
Trassi (cooked), plum brandy, almond (roasted, *Prunus amygdalus*)	9
Macadamia nut (roasted, *Macadamia integrifolia*), sesame seed (roasted), mango (raw)	0–0.1
Mango (canned), cauliflower (cooked), tamarind (*Tamarindus indica*), pear brandy	7
Apple brandy, beetroot (cooked),artichoke (cooked, *Cynarus scolymus* L.), gin, rice bran, traditional rice (cooked), quince (*Cydonia oblonga*), radish (fermented), shoyu (fermented soya hydrolysate), bacuri (*Platonia insignis*), cupuacu (*Theobroma grandiflora*), muruci (*Brysonima crassifolia*), potato (sweet, heated), sukiyaki, licorice (*Glycyrrhiza glabra* L.), matsutake (*Tricholoma matsutake*), strawberry wine, pumpkin (*Cucurbita pepo* L.), sake, oat groats, maize, cashew apple (*Anacardium occidentale*), basil (*Ocimum basilicum*), malt, peated malt, wort, bonito (dried, Katsuobishi), elderberry (*Sambucus nigra* L.), mangosteen (*Garcinia mangostana*), cherimoya (*Annona cherimola*), bilberry wine, buchu oil, vanilla, mountain papaya (*Carica pubescens*)	< 0.01
Wild rice (*Zizania aquatica*), chicory (*Cichorium intrybus* L.), endive (*Cichorium endivia* L.), ouzo	0–0.2
Sapodilla fruit (*Achras sapota* L.)	trace
Aubergine (*Solanum melongena* L.), pistachio nut (roasted, *Pistachia vera*), arrack	17.2
Nectarine	< 0.01

*CIVO–TNO, 1994.

probably by the supply of endogenous glycine (Gregus *et al.*, 1993).

In rodents, the principal metabolite furoic acid may also be metabolized by oxidation of the heteroaromatic ring. Since heteroaromatic and aromatic carboxylic acids do not normally decarboxylate *in vivo* (Caldwell, 1982), it can be assumed that oxidation of the furan ring system of furoic acid precedes the loss of CO_2. Epoxidation (Ramsdell and Eaton, 1990) or hydroxylation (Koenig and Andreesen, 1990; Ravindranath and Boyd, 1985) of the furan ring may yield reactive intermediates (e.g. furfural-2,3-epoxide, acetylacrolein or α-ketoglutaric acid) which readily decarboxylate. Biochemical changes in the lungs and livers of animals exposed to furfural indicate that ring oxidation may be catalysed by a cytochrome-*P*450b (CYP450b) isoenzyme yielding an intermediate which subsequently conjugates with glutathione (Gupta *et al.*, 1991; Mishra *et al.*, 1991).

2. *Humans*

As in laboratory animals, the predominant pathway for detoxication of furfural in man is oxidation of the aldehyde to yield furoic acid which may either conjugate with amino acids or condense with acetyl coenzyme A (CoA) to produce the furanacrylic acid. There is no evidence to suggest that

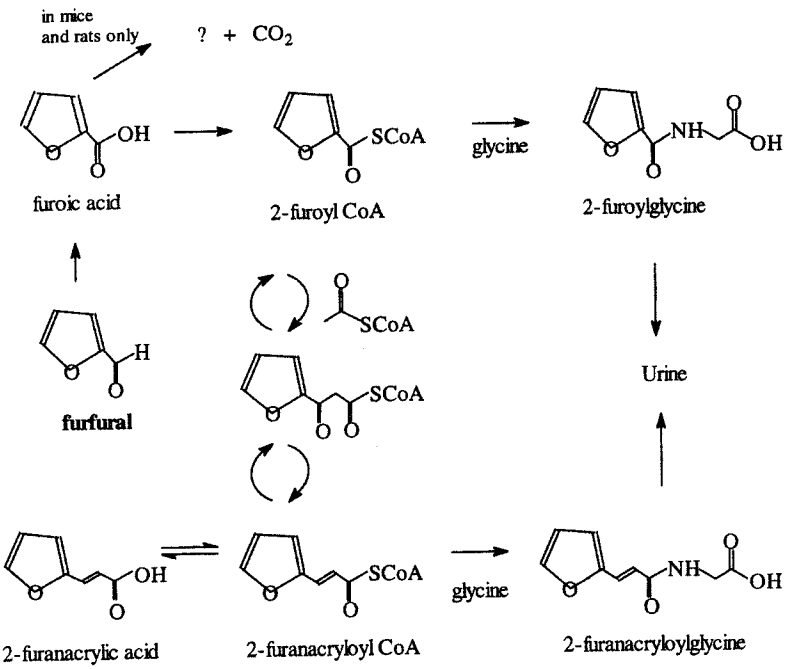


Fig. 2. The metabolism of furfural in humans and rodents.

oxidation of the furan ring occurs in humans as it does in rodents.

Results of inhalation and dermal absorption studies indicate that furfural is efficiently and rapidly absorbed in humans through the lungs and skin (approx. 20% of the amount absorbed by the lungs) (Flek and Sedivec, 1978). When six volunteers were exposed to 30 mg furfural/m^3 for 8 hr, mean pulmonary retention was reported to be 77.9% (range 74.9–82.1%). Retention was independent of the concentration of furfural vapour and the time of exposure. An average 8-hr exposure maximum was equivalent to a dose level of 1.9 mg/kg. The biological half-life of absorbed furfural in humans is short, approximately 2 hours. Essentially 100% of the absorbed furfural could be accounted for: 96.9% (range 92.75–102.6%) was oxidized to 2-furoic acid and excreted as the glycine conjugate; 0.5–5% was excreted as furanacrylic acid; and less than 1% was exhaled unchanged. No evidence of decarboxylation was observed in humans at levels of exposure (approx. 2 mg/kg) (Flek and Sedivec, 1978) similar to those producing CO_2 in female mice (0.1 mg/kg) (Parkash and Caldwell, 1994) or in rats (12.5 mg/kg) (Nomeir *et al.*, 1992).

IV. Toxicology

A. Acute and short-term toxicity

Based on oral LD_{50} values in various species, the relative order of the acute oral toxicity of furfural is rat > mouse > dog. The range of oral LD_{50} values is 50 mg/kg to 149 mg/kg in the rat (Deichmann and Gerarde, 1969; Fassett, 1963; Jenner *et al.*, 1964; Kuznetsov, 1966), 250 mg/kg to 500 mg/kg in the mouse (Fassett, 1963; Klucik *et al.*, 1961; Kuznetsov, 1966; NTP, 1990a) and 650 mg/kg to 950 mg/kg in the dog (Deichmann and Gerarde, 1969; Fassett, 1963).

In rodents, acute effects are reported mainly in the liver. Rats administered a single 50 mg/kg dose of furfural by gavage exhibited sporadic eosinophilic degeneration of hepatic cells with nuclear pyknosis along with eosinophilic necrosis and increased hepatocyte mitosis. No massive or zonal necrosis was observed after a single dose (Shimizu and Kanisawa, 1986). Changes in cytosolic and mitochondrial enzyme activities are indicative of increased hepatocellular catabolism (Jonek *et al.*, 1975; Kaminska, 1977).

B. Subchronic studies

In oral subchronic studies, dose levels of furfural greater than 50 mg/kg/day are primarily associated with hepatic effects. Rats were fed furfural at a concentration of 20 ml/kg in the diet (approx. 175 mg/kg/day) for 1 week, then 30 ml/kg in the diet for the second week, and then 40 ml/kg in the diet (approx. 350 mg/kg/day) from days 15 to 90. From days 90 to 120 rats were given the diet containing 40 ml/kg for only 5 days per week to prevent a reduction in weight gain. The concentration of furfural in the feed was not analysed after preparation of the diet (Shimizu and Kanisawa, 1986). Therefore, the actual intake level of furfural is unknown but may have been significantly lower because the furfural-containing diet was prepared only once at the initiation of the study.

Rats killed after 90 days exhibited marked cholangiofibrosis with areas of increased density containing red nodules. The nodules showed a fibrous widening of Glisson's sheath, bile duct proliferation, and destruction of the limiting plates. Parenchymal

damage consisted of bridging necrosis and hydropic degeneration of hepatocytes. Parenchyma often showed no cirrhotic changes in areas other than those with fibrosis suggesting a regenerative process in the liver. Increased numbers of cells undergoing mitosis was evident. In rats killed on the 120th day of the study, similar but more prominent findings were reported. The incidence of hepatic fibrosis increased in the second group of rats. No unusual hepatocyte growth was noted. Furfural treatment did not result in any hyperplastic changes in the liver (Shimizu and Kanisawa, 1986).

In a study designed to examine the mechanism of furfural induced-hepatocyte injury, groups of male rats were maintained on diets containing furfural at levels calculated to result in the average daily intake of 20 mg/kg for the first 30 days and 30 mg/kg/day thereafter. Furfural-containing diets were terminated after 15, 30, 60, 90, 120 or 150 days and animals were killed 14 days later. An increase in the duration of exposure was accompanied by increased number and size of glutathione *S*-transferase (GST)-P positive foci in rat hepatocytes (Shimizu *et al.*, 1989). GST-P has been reported to be a good marker for early detection of preneoplastic or neoplastic cells (Pickett and Lu, 1988). These data suggest that furfural has potential for inducing toxicity that may produce a neoplastic response.

Rats and mice were administered furfural in corn oil daily by gavage, 5 days a week for 13 weeks at dose levels of 0, 11, 22, 45, 90 or 180 mg/kg for rats and 0, 75, 150, 300, 600 or 1200 mg/kg for mice. Low survival rates were reported at the highest dose level in male rats and the two highest dose levels in male and female mice. Relative and absolute liver and kidney weights in male rats dosed with 90 mg/kg/day or more were significantly higher than those in control rats. A significant increase in relative liver weight was measured in mice administered a dose level of approximately 75 mg/kg/day. An increase in the incidence of cytoplasmic vacuolization, primarily in the centrilobular regions, was observed in the hepatocytes of treated male rats compared with the control group (vehicle control, 4/10; 11 mg/kg, 10/10; 22 mg/kg, 10/10; 45 mg/kg, 10/10; 90 mg/kg, 9/10). However, the severity of the effect (i.e. minimal to mild) was similar for control and treated male rats. A dose-related increase in centrilobular coagulative necrosis of hepatocytes was observed in mice treated with 150 mg/kg/day or more. Inflammation of the liver with minimal to mild mononuclear inflammatory cell infiltrate was also present. No lesions were observed in the kidneys of these rats or mice (NTP, 1990a).

C. Chronic studies

Studies have been designed to evaluate the effect of furfural on the carcinogenic potential of benzo[*a*]pyrene (BaP) and diethylnitrosamine. In the first study, Syrian golden hamsters were provided 0.2 ml 1.5% furfural in physiological saline solution (approx. 25 mg/kg), 0.5% BaP (8.3 mg/kg body weight), or 1.5% furfural plus 0.5% BaP intratracheally once weekly in a 36-week study. No respiratory tract tumours or peritracheal tumours were found. No tumorigenic effects were observed by the administration of furfural alone (Feron, 1972). In a subsequent inhalation study, the effect of furfural on the carcinogenic potential of BaP and diethylnitrosamine was evaluated (Feron and Kruysse, 1978). Male and female hamsters were exposed to atmospheres containing 400 ppm (1550 g/m^3) furfural 7 hours per day, 5 days per week for 9 weeks, then 330 ppm (1280 mg/m^3) for 11 weeks, and 250 ppm (970 mg/m^3) for an additional 32 weeks. Respiratory tract effects included atrophy and downward growth of the olfactory epithelium, degenerative changes in Bowman's glands, and the appearance of cyst-like structures in the lamina propria beneath the olfactory epithelium. Although furfural exposure produced irritant effects on the olfactory epithelium, treatment did not result in any evidence of liver toxicity or carcinogenicity. Localized irritant effects of the nasal cavity and respiratory tract and the absence of hepatic effects have been observed when B6C3F$_1$ mice and F344/N rats were exposed to atmospheres containing 0, 2, 4, 8, 16 or 32 ppm of the metabolic precursor furfuryl alcohol for 13 weeks (Mellick *et al.*, 1991).

D. 2-Year carcinogenicity NTP bioassay

1. Rats. Male and female F344/N rats were administered furfural in corn oil by gavage daily at dose levels of 0, 30 or 60 mg/kg, 5 days per week for 2 years (NTP, 1990a). Furfural treatment had no effect on body weight or survival. An increased incidence of centrilobular hepatocellular necrosis was observed in treated males (control, 3/50; low-dose, 9/50; high-dose 12/50). Focal cystic degeneration also was observed in high-dose males. There was no evidence of carcinogenicity in female rats at any dose level. In male rats, 2/50 high dose (60 mg/kg/day) animals developed cholangiocarcinomas and two other animals had bile duct dysplasia, which was said to provide some evidence of carcinogenicity based on the rarity of these lesions in F344/N rats (historical incidence, 3/2145). When evaluating the relevance of this conclusion to human safety, additional factors should be considered.

First, female rats showed no evidence of carcinogenicity at any dose level. Males exhibited lesions only at the high dose (60 mg/kg/day), but the incidence (2/50) of this lesion was not statistically significant and, while of interest, cannot be said to have any biological significance, given the marked toxicity exerted by the substance. Secondly, it was observed that the livers of nearly all of the male rats in all groups were abnormal, and it was not clear whether the finding of bile duct hyperplasia in controls (90%) as well as treated animals (82 and 84%) was a result of the corn oil gavage. In view of this finding, the biological significance of the cholangiocarcinoma must be assessed in the context of co-existing non-neoplastic liver lesions.

Table 3. Incidences of hepatocellular adenomas and carcinomas in male and female B6C3F$_1$ mice administered furfural by gavage[1]

	Control	50 mg/kg	100 mg/kg	175 mg/kg
Male mice				
Hepatocellular adenoma	9/50	13/50	11/49	19/50
Hepatocellular carcinoma	7/50	12/50	6/49	21/50
Combined rates[2]	16/50	22/50	17/49	32/50
Fisher's Exact Test (combined)		$P = 0.151$	$P = 0.472$	$P = 0.001$
Female mice				
Hepatocellular adenoma	1/50	3/50	5/50	8/50
Hepatocellular carcinoma	4/50	0/50	2/50	4/50
Combined rates[2]	5/50	3/50	7/50	12/50
Fisher's Exact Test (combined)		$P = 0.357$	$P = 0.380$	$P = 0.054$

[1] NTP (1992).
[2]Combined Rates—Total number of animals with adenomas or carcinomas.

Thirdly, the 30 mg/kg dose level for rats that produced no carcinogenic effects in the 2-year NTP study is greater than 10,000 times the estimated daily *per capita* intake ("eaters only") of 2 μg/kg from use of furfural as a flavour ingredient and is approximately 100 times the total intake of furfural (0.3 mg/kg/day) from natural and other sources.

Fourthly, the lack of any hepatotoxic effects in hamsters administered furfural either orally for 36 weeks at a dose level of 25 mg/kg or by inhalation for 1 year at concentrations of 970–1550 mg/m^3 is not consistent with the report of hepatotoxicity in rats in the NTP bioassay. Additionally, a metabolic pathway (i.e. furan ring oxidation to yield a reactive intermediate) may be an important component of the mechanism for hepatotoxicity in rodents that is not seen in humans.

Other aspects of the results of the NTP study are relevant to the overall evaluation and provide insight into the mechanism of the hepatic response in male rats. The incidence of centrilobular necrosis in all groups of male rats (4%, control; 16%, 30 mg/kg/day; 24%, 60 mg/kg/day) was accompanied by a high incidence of bile duct hyperplasia (90%; 82%; 84%), basophilic foci (74%; 70%; 52%), and clear foci (42%; 38%; 32%). Necrosis and bile duct hyperplasia are counterparts to and often precede neoplastic development in the liver of rodents (Newberne *et al.*, 1987). The fact that these lesions, particularly bile duct hyperplasia, were present in the vehicle-control group indicates that there were background anomalies contributing to the overall results. Since basophilic and clear cell foci precede and accompany neoplasia in the liver of rodents, the high incidence of basophilic (74%) and clear cell foci (42%) in untreated males further supports the likelihood of confounding factors in the study (Butterworth *et al.*, 1992).

Based on these observations it can be concluded that high dose levels of furfural are hepatotoxic (cytotoxic) and increase hepatocyte proliferation and cell death. This response is similar to that observed with other non-genotoxic agents such as chloroform (Reitz *et al.*, 1990) and carbon tetrachloride in the liver, *d*-limonene in the kidney (Flamm and Lehman-McKeeman, 1991), and saccharin in the urinary bladder (Cohen and Ellwein, 1988).

2. Mice. Male and female B6C3F$_1$ mice were administered furfural in corn oil by gavage at daily dose levels of 0, 50, 100 or 175 mg/kg, 5 days per week for 2 years. One male mouse in both the mid- and high-dose groups, and one low-dose female were found to have a renal cortical adenoma. One low-dose male was found to have a renal cortical carcinoma. In the evaluation, the NTP commented that "renal cortical neoplasms were also said to have been observed in male mice". In evaluating the report that renal neoplasms were observed in furfural-exposed mice, the following factors must be considered: the high incidence of age-related renal lesions in both control and treated-groups of mice; the fact that the number of neoplasms (males; control, 0/50; 50 mg/kg/day, 1/50; 100 mg/kg/day, 1/49; 175 mg/kg/day, 1/50: females; control, 0/50; 50 mg/kg/day, 1/50; 100 mg/kg/day, 0/50; 175 mg/kg/day, 0/50) are not statistically significant; the lack of a dose–response relationship; and the lack of non-neoplastic or preneoplastic renal pathology. These considerations and the fact that the cortical tumours occurred sporadically indicate that they were of spontaneous origin.

A marginal increase in the incidence of hyperplasia and papillomas in the forestomach of female mice was considered to be of no biological significance. These observations were probably related to the irritative effects of the compound when administered chronically by gavage or the gavage method of administration itself. Furthermore, none of the animals showed evidence of malignant forestomach lesions.

In male mice, an increase in the incidence of hepatocellular adenomas and carcinomas was statistically significant only in the high-dose group (16/50, controls; 22/50, 50 mg/kg/day; 17/50, 100 mg/kg/day; 32/50, 175 mg/kg/day) (Table 3). In female mice, an increase in the incidence of hepatocellular adenomas was also deemed by the NTP to be statistically significant only in the high-dose group (1/50 for controls and 8/50 for 175 mg/kg/day group). The incidence of carcinomas was not increased (4/50 for control and 4/50 for 175 mg/kg/day group)

(NTP, 1990a). The NTP concluded that there was *clear* evidence of carcinogenic activity for male $B6C3F_1$ mice based on increased incidence of hepatocellular carcinomas and adenomas, and there was *some* evidence of carcinogenic activity in female $B6C3F_1$ mice based on increased incidence of hepatocellular adenomas.

The data pertaining to the carcinogenicity of furfural in mice is presented in Table 3. These data require evaluation both from a statistical and biological perspective. There is no convincing statistical evidence that the incidence of lesions in female mice was related to administration of furfural. Liver neoplasms occur at a high and variable spontaneous incidence in both sexes of the $B6C3F_1$ mouse. According to guidelines proposed by Haseman *et al.* (1986), a compound is considered to exhibit carcinogenic potential if the highest dose is associated with an increased incidence of a common tumour that is significant at the 1% ($P < 0.01$) level, or an increased incidence in a rare tumour (frequency less than 1%) at the 5% ($P < 0.05$) level. Consistent with this proposal, it is the view of the FEMA Expert Panel that a significance level of 1% ($P < 0.01$) be employed in the statistical analysis of the mouse liver tumour data in the light of the high background incidence of hepatic tumours in mice. While the incidence of liver tumours in high-dose male mice in the NTP study was significant (Fisher's Exact Test for pairwise comparisons) at the 0.1% ($P = 0.001$) level, in the female mice it was significant only at the 5.4% ($P = 0.054$) level. Therefore, the incidence of hepatocellular adenomas is not statistically significant for the high dose group of female mice.

The only other indication that furfural possesses carcinogenic potential is the report of liver tumours in the male mouse at the highest dose level (175 mg/kg), a level associated with extensive evidence of hepatotoxicity. It should be noted that this dose is greater than 30,000 times the daily *per capita* intake ("eaters only") of approximately 2 μg/kg from use of furfural as a flavour ingredient, and is at least 500 times the total intake of 0.3 mg/kg/day from exposure to furfural and furfural precursors as flavour ingredients and as natural components of food.

The high-dose male mice showed a significant increase in the incidence of multifocal necrosis of the liver, not seen in the female mice at any dose level, indicating that the incidence of carcinomas in high-dose male mice was associated with chronic hepatic toxicity. This conclusion is supported by the observation that there was no incidence of carcinoma and no hepatocellular necrosis in either low-dose males or treated females. The lower incidence and milder nature of the hepatotoxic effects, and the lack of a significant increase in hepatic tumours in the mid-dose group of male mice is additional evidence that the carcinogenic response in the high-dose group is related to chronic hepatic toxicity. Therefore, it can be concluded that the carcinogenic potential in this sensitive breed and sex of laboratory rodent is a secondary biological response to dose-dependent hepatotoxicity.

The dose level of 50 mg/kg that produced no significant toxic or carcinogenic effects in mice in the 2-year NTP study is greater than 10,000 times the daily *per capita* intake ("eaters only") of approximately 2 μg/kg/day from use of furfural as a flavour ingredient, and is at least 100 times the total intake of 0.3 mg/kg/day from use of furfural and furfural precursors as flavour ingredients and as natural components of food.

E. Mutagenicity and genotoxicity studies

1. *In vitro.* Mutagenicity testing of furfural *in vitro* at concentrations up to those levels causing cytotoxicity has shown no evidence of mutagenicity in *Salmonella typhimurium* (SAL) strains TA98, TA102, TA104, TA1535 and TA1537 nor in two strains of *Escherichia coli* with or without metabolic activation (Kato *et al.*, 1989; Loquet *et al.*, 1981; McMahon *et al.*, 1979; Marnett *et al.*, 1985; Mortelmans *et al.*, 1986; Nakamura *et al.*, 1987; Sasaki and Endo, 1978; Shane *et al.*, 1988; Zdzienicka *et al.*, 1978). Furfural showed no mutagenic potential in a *rec* assay using *Bacillus subtilis* in a direct streaking method (Osawa and Namiki, 1982). Furfural incubated with SAL strain TA100 was reported to be non-mutagenic in some reports (Kato *et al.*, 1989; McMahon *et al.*, 1979; Sasaki and Endo, 1978; Shane *et al.*, 1988), and weakly mutagenic in others (Loquet *et al.*, 1981; Mortelmans *et al.*, 1986; Zdzienicka *et al.*, 1978).

Furfural produced an increased frequency of chromatid breaks and exchanges in Chinese hamster ovary (CHO) cells in a dose-dependent manner with or without metabolic activation. Increased chromatid breaks and exchanges were reported at the lowest concentration (2.5 mM) with metabolic activation only (Stich *et al.*, 1981a,b). An increased frequency of sister chromatid exchanges (SCE) was reported when human lymphocytes were incubated with furfural. However, furfuryl alcohol did not induce an increase in SCEs with or without metabolic activation (Gomez-Arroyo and Souza, 1985).

Furfural induced an increase in trifluorothymidine resistance when incubated with L5178Ytk +/− mouse lymphoma cells (MLA) at solution concentrations of 200 and 400 μg/ml without metabolic activation, but showed no mutagenic activity at 25, 50 or 100 μg/ml (McGregor *et al.*, 1988). Concentrations of 400 and 800 μg/ml were cytotoxic in separate trials.

The disparity of positive and negative results observed for furfural in the four widely used *in vitro* genotoxicity tests (SAL, MLA, ABS and SCE) is a common trend. Consequently, the utility of these assays for predicting the carcinogenic potential of substances in long-term rodent assays has been thoroughly evaluated. Based, in part, on the following conclusions by researchers affiliated with the NTP (Haseman *et al.*, 1990; Zeiger *et al.*, 1990), the use of ABS, SCE and MLA assays to prioritize

substances for carcinogenicity testing has been curtailed within the NTP: (1) There appears to be no evidence of complementarity among the four genotoxicity assays; (2) the results of a battery of tests have not been shown to be more conclusive than the use of the SAL assay alone (Smart, 1994); and (3) the disparity of positive and negative results in selected assays precludes the formation of conclusions on the potential carcinogenicity of a substance.

Moreover, the genetic endpoints [i.e. sister chromatid exchanges (SCE) and chromosome aberrations (ABS) of Chinese hamster ovary (CHO) tests] may be artefacts resulting from lysosome breakdown secondary to cytotoxicity, and not from the direct action of the test substance on DNA. These genetic effects may result at concentrations of test substance that produce high levels of cytotoxicity, involving lysosomal breakdown and release of DNAase which induces chromosome aberrations and DNA double-strand breaks (DSBs) (Bradley *et al.*, 1987; Zajac-Kaye and Ts'o, 1984). Cells with DSBs that survive could be subject to a variety of genotoxic consequences including chromosome breaks and rearrangements. Although an evaluation of cytotoxicity and lysosomal breakdown was not included in furfural-related SCE and ABS studies (Stich *et al.*, 1981a,b), furfural was cytotoxic at similar concentrations in SAL assays (Marnett *et al.*, 1985; Shane *et al.*, 1988). Data such as those resulting from the Chinese hamster ovary cytogenetics assays are difficult, if not impossible, to interpret in light of the effect of lysosomal breakdown and cytotoxicity.

The positive result obtained in a MLA was at near-lethal concentrations. The results of the MLA for simple aliphatic and aromatic substances have been shown to be inconsistent with the results of other standardized genotoxicity assays (Heck *et al.*, 1989). Culture conditions of low pH and high osmolality, which may occur on incubation with substances (aldehdyes, carboxylic acids, lactones) having a potentially acidifying influence on the culture medium, have been shown to produce false-positive results in this and other assays (Heck *et al.*, 1989).

Considering the results of the SAL assay, the weight of evidence suggests furfural is not genotoxic. The few reports of weak mutagenicity of furfural in a single strain of SAL (TA100) are not supported by negative results in other strains (TA98, TA102, TA1535, TA1537), especially TA104, a strain more sensitive to carbonyl mutagenesis (Marnett *et al.*, 1985; Shane *et al.*, 1988). The principal metabolite of furfural, furoic acid, exhibited no evidence of mutagenicity in SAL strain TA100 (DeFlora, 1978). In addition to the results of these *in vitro* tests, results of *in vivo* and molecular-based assays (e.g. *in vivo–in vitro* unscheduled DNA synthesis) support the conclusion that furfural is not genotoxic.

2. *In vivo*. Furfural did not induce sex-linked recessive lethal mutations or reciprocal translocations in male *Drosophila melanogaster* when incorporated into the diet at 1000 ppm. Mutations were induced when furfural was administered by injection at a concentration of 100 ppm, but no reciprocal translocations occurred. The same pattern of responses were reported for other simple aldehydes (e.g. acetaldehyde and *trans*-cinnamaldehyde) by the intraperitoneal (positive) and oral (negative) modes of administration (Woodruff *et al.*, 1985). No chromosomal loss was observed when male *Drosophila* were fed or injected with furfural at concentrations approximately 25 to 33% of the lethal dose and then mated with repair-proficient females. Chromosomal loss was observed when treated males were mated with repair-deficient females (Rodriquez-Arnaiz *et al.*, 1992). No sex-linked recessive lethal mutations or sex-chromosomal losses were reported when male *Drosophila* were injected with furfuryl alcohol (Rodriquez-Arnaiz *et al.*, 1989).

In vivo mammalian tests with furfural have produced mainly negative results. Furfural did not induce SCE or chromosome aberrations in B6C3F$_1$ mouse bone marrow cells following intraperitoneal injections of doses ranging from 50 to 200 mg/kg (NTP, 1990a) or in human lymphocytes from workers occupationally exposed to furfural and furfuryl alcohol (Gomez-Arroyo and Souza, 1985). No genotoxic effects or spermhead abnormalities were reported when mice were given up to 4000 ppm furfural daily for 5 weeks (Subramanyam *et al.*, 1989).

Furfural was administered to male F344/N rats in corn oil by gavage at dose levels of 5.0, 16.7 or 50 mg/kg. Hepatocytes isolated 2 or 16 hr after administration failed to exhibit any evidence of unscheduled DNA synthesis at any of the three dose levels (Phillips *et al.*, 1997). Furfural treatment was reported to reduce the amount of double-stranded DNA in alkaline-treated calf thymus DNA (Hadi *et al.*, 1989). Although furfural induced deletions, single strand breaks and alterations when incubated with plasmid DNA, repair of the damaged plasmids occurred on propagation in the host (Khan and Hadi, 1993).

It is concluded that the value of the report that furfural induces sex-linked lethal mutations when injected into *Drosophila* (Rodriquez-Arnaiz *et al.*, 1992) is not significant in the safety evaluation of furfural when compared with the observations that furfural did not induce mutations when fed in the diet at higher levels; furfuryl alcohol did not induce mutations when injected into *Drosophila* at higher levels; and there is no evidence of genotoxicity in *in vivo* studies in bone marrow cells of mice and lymphocytes of humans.

The difference between the positive *in vitro* and negative *in vivo* results for furfural is not surprising in light of the recognized reactivity of aldehydes (see Chemical Characteristics, Section II and Metabolism, Section IV). Furfural exhibits a short

plasma half-life and is efficiently metabolized to furoic acid, which is conjugated with glycine *in vivo*. These are important *in vivo* conditions that are difficult to establish in the above mentioned *in vitro* assays. Sporadic positive responses of the *in vitro* tests are not supported by more relevant findings of *in vivo* studies in which furfural undergoes efficient metabolic detoxication. The observation that furfural is not a genotoxic carcinogen in *in vivo* studies in mice, rats or humans is consistent with its rapid metabolic conversion to the glycine conjugate of furoic acid in each of the three species.

Like furfural, isobutyraldehyde (NTP, 1996) and benzaldehyde (NTP, 1990b) are reactive aldehydes which are efficiently metabolized to their corresponding acid and show positive results in the selective *in vitro* genotoxicity assays (positive AMS, MLA, SCE and ABS for isobutyraldehyde and positive MLA and SCE for benzaldehyde). However, they were negative *in vivo* in the sex-linked lethal mutation assay in *Drosophila* (NTP, 1990b and 1996) and in the micronucleus test for mice and rats (NTP, 1996). The observations that these aldehydes are not genotoxic *in vivo* are confirmed by the results of the 2-year carcinogenicity bioassays. Isobutyraldehyde was not carcinogenic in a 2-year bioassay in either sex of mice or rats (NTP, 1996). Benzaldehyde was not carcinogenic in rats, and the benign lesions observed in the forestomach of mice (NTP, 1990b) were concluded to be the result of forestomach epithelia being chronically in contact with an irritating, concentrated mixture of test article and vehicle (corn oil) administered daily by gavage (NTP, 1990b; Smith and Ford, 1993).

On a molecular level, the observation that furfural-induced alterations, single strand breaks and deletions in DNA are repaired by the host, supports the Panel's conclusion that furfural exhibits little potential for significant interaction with DNA and genotoxicity. The negative results of the rat *in vivo–in vitro* UDS study provides additional evidence that furfural does not act directly on DNA.

Taking into account the results of the NTP bioassay (NTP, 1990a,b) and genotoxicity tests, furfural is not genotoxic. Rather, the effects of furfural result from a non-genotoxic mechanism of action in which high dose levels are hepatotoxic in male $B6C3F_1$ mice, leading to cell proliferation and cell death and, after prolonged exposure, liver tumours.

F. Oncogene activation studies

The mechanism of tumour induction in mouse liver was studied by examining furfural-induced tumours in $B6C3F_1$ mice (NTP, 1990a). The authors reported that the pattern of mutations in the oncogene of liver tumours that occurred spontaneously in control animals differed from the pattern of those that occurred in some furfural-treated animals. In the spontaneously formed tumours in control animals, activated *ras* genes were H-*ras* in 15 of 17 tumours (one *raf* and one unknown oncogene) with activating point mutations at codon 61. In the furfural-induced tumours, 9/10 were H-*ras* (1 K-*ras* oncogene) with activating point mutations occurring at codon 61 in six tumours, codon 13 in two tumours, and codon 117 in one tumour. Based on the observation of 'atypical' oncogene activation (i.e. non-H-*ras*, or H-*ras* with a mutation other than at codon 61) in the liver tumours of the furfural-treated mice, the authors suggested that furfural induces tumours through a direct genotoxic mechanism. According to the authors, the lack of cytotoxicity observed in the furfural-treated groups on histopathological examination after 90 days of treatment further suggests that tumour formation occured by a genotoxic pathway (Reynolds *et al.*, 1987).

Other data indicate that the neoplastic effect in the livers of mice treated with furfural proceeds by a non-genotoxic mechanism with cytotoxicity preceding tumour formation. First, furfural is not directly genotoxic in a battery of well-validated *in vitro* tests (see above) nor does it induce unscheduled DNA synthesis *in vivo* in male rats (Phillips *et al.*, 1997). Secondly, the pattern of oncogene activation (H-*ras* with a mutation at codon 61) in the majority of tumours was the same for both the furfural-treated and control groups of mice. Furthermore, the same pattern of oncogene activation has been reported in 24 of 25 mouse liver tumours induced by potent genotoxic carcinogens (Wiseman *et al.*, 1986), suggesting that there is no direct correlation between the pattern of oncogene activation in these experiments and the mechanism of tumour formation. Thirdly, with regards to cytotoxicity, there was a significant incidence of liver toxicity (e.g. multifocal necrosis, multifocal chronic inflammation of the subserosa, and pigmentation of the subserosa) in the high-dose group of male mice which exhibited a high incidence of liver tumours.

Overall, there is no conclusive evidence for furfural-induced hepatic carcinogenicity by direct interaction of furfural with DNA. It is possible that in the NTP study, furfural acted as a promoter of a subpopulation of hepatocytes bearing a spontaneously mutated *ras* gene. The data suggest that furfural-induced hepatic carcinogenicity is confounded by co-existing toxicity and the effects occurred only in the male mouse at daily gavage dose levels greater than 1000 times the estimated level of exposure to furfural by humans. As compared with humans, hepatotoxicity may be more prevalent in rodent species in which metabolic intoxication pathways (i.e. furan ring oxidation) are favoured.

V. Recognition of GRASr status

Furfural was determined to be generally recognized as safe (GRAS) under conditions of intended use as a flavour ingredient by the FEMA Expert Panel in 1965. In 1975, the Panel evaluated the available data and affirmed the GRAS status of this

flavour ingredient (GRASa). In 1993, the Panel initiated a comprehensive program to re-evaluate the status of all FEMA GRAS flavour ingredients concurrent with a systematic revision of the FEMA Scientific Literature Reviews (SLRs). Furfural was reaffirmed as GRAS (GRASr) in 1995 based on its mode of metabolic detoxication in humans; its low level of flavour use; the safety factor calculated from results of subchronic and chronic studies; the lack of genotoxic and mutagenic potential and the conclusion that the only statistically significant finding in the NTP studies—an increased incidence of hepatocellular adenomas and carcinomas in the high dose group of male mice—was secondary to pronounced hepatotoxicity and does not indicate any risk to human health under conditions of use as a flavour ingredient. This evidence of safety is supported by the occurrence of furfural as a natural component of traditional foods, at concentrations in the diet resulting in a 'natural intake' that is at least 100 times higher than that which occurs when furfural is employed as a flavour ingredient.

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