NITROFURAL (NITROFURAZONE)

This substance was considered by a previous Working Group, in June 1974, under the title 5-nitro-2-furaldehyde semicarbazone (IARC, 1974). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

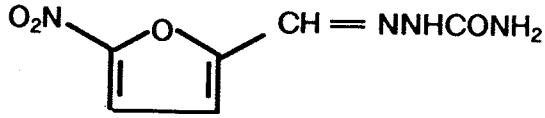
1.1 Synonyms

Chem. Abstr. Services Reg. No.: 59-87-0

Chem. Abstr. Name: Hydrazinecarboxamide, 2-[(5-nitro-2-furanyl)methylene]-

Synonyms: 2-Furancarboxaldehyde; 5-nitrofuraldehyde semicarbazide; nitrofuraldehyde semicarbazone; 5-nitro-2-furaldehyde semicarbazone; 5-nitrofuran-2-aldehyde semicarbazone; 5-nitro-2-furancarboxaldehyde semicarbazone; 5-nitro-2-furfuraldehyde semicarbazone; 5-nitrofurfural semicarbazone; 5-nitro-2-furfural semicarbazone; (5-nitro-2-furfurylideneamino)urea; 1-(5-nitro-2-furfurylidene)semicarbazide

1.2 Structural and molecular formula and molecular weight



$C_8H_6N_4O_4$ Mol. wt: 198.14

1.3 Chemical and physical properties of the pure substance

From Windholz (1983) and Reynolds (1989)

(*a*) *Description*: Pale-yellow needles

(*b*) *Melting-point*: 236-240°C (decomposition)

(*c*) *Solubility*: Very slightly soluble (1:4200) in water at pH 6.0-6.5, soluble in alkaline solutions; slightly soluble in ethanol (1:590), propylene glycol (1:350), acetone (1:415), dimethylformamide (1:15) and polyethylene glycol (1:86); almost insoluble in chloroform (1:27000) and benzene (1:43500)

(*d*) *Spectroscopy data*: Infrared and ultraviolet spectra have been reported.

(*e*) *Stability*: Stable in solid state at less than 40°C when protected from light; darkens with prolonged exposure; discolours on contact with alkali

1.4 Technical products and impurities

Trade names: Acutol; Aldomycin; Alfucin; Amifur; Babrocid; Becafurazona; Becafurazone; Biofuracina; Biofurea; Chemofuran; Chixin; Cocafurin; Coxistat; Dermofural; Dymazone; Eldezol F-6; Fedacin; Flavazone; Fracine; Furacilin; Furacilinum; Furacillin; Furacin; Furacin-E; Furacine; Furacinetten; Furacin-HC; Furacoccid; Furacort; Furacycline; Furalcyn; Furaldon; Furalone; Furametral; Furan-ofteno; Furaplast; Furaseptyl; Furaskin; Furaziline; Furazin; Furazina; Furazol W; Furazone; Furesol; Furfurin; Furosem; Fuvacillin; Germex; Hemofuran; Ibiofural; Mammex; Mastofuran; Monafuracin; Nefco; NF-7; NFS; Nfz mix; Nifucin; Nifurid; Nifuzon; Nitrazone; Nitreofural; Nitrofurastan; Nitrofurazan; NSC-2100; Nitrozone; Otofural; Otofuran; Rivafurazon; Rivopon-S; Sanfuran; Spray-Dermis; Spray-foral; Vabrocid; Veterinary nitrofurazone; Yatrocin

Nitrofural has been reported to contain 3% 5-nitro-2-furaldehyde azine as an impurity (Morris *et al.*, 1969). It is available in the USA as creams, ointments, powders, solutions, sprays, suppositories and surgical dressings (Barnhart, 1989).

2. Production, Occurrence, Use and Analysis

2.1 Production and occurrence

The action of nitrofural as a topical antibacterial agent was first reported in the USA in 1944 (Dodd & Stillman, 1944), and the product was available for general use in 1945 (Miura & Reckendorf, 1967). Commercial production in the USA was first reported in 1955 (US Tariff Commission, 1956).

Nitrofural can be prepared by the reaction of 5-nitrofurfural with an aqueous solution of a mixture of semicarbazide hydrochloride (see IARC, 1987) and sodium acetate (Stillman & Scott, 1947). It can also be synthesized from the reaction of acetone semicarbazone or other semicarbazones with 5-nitrofurfuraldoxime (Gever & O'Keefe, 1960). It is synthesized in China, Hungary, India, Mexico and Spain (Chemical Information Services, 1989-90).

Nitrofural is not known to occur naturally.

2.2 Use

Nitrofural is a broad-spectrum bactericidal (Chamberlain, 1976). It also has antiprotozoal and antiparasitic activities (Reynolds, 1982).

Nitrofural is used locally for the treatment of wounds, burns, ulcers and skin infections; it has also been applied locally to the ear, eye and bladder. Nitrofural is used as a coccidiostatic and antibacterial agent in farm animals, administered in water or feed (Anon., 1979; Reynolds, 1989).

Oral administration has been restricted to the treatment of late-stage African trypanosomiasis that is refractory to melarsoprol. The dosage for adults is 500 mg three or four times daily for five to seven days. In addition, it has been given orally in doses of 100 mg four times daily for five to six days in the treatment of acute bacillary dysentery (Reynolds, 1982).

2.3 Analysis

Nitrofural has been analysed in pharmaceutical preparations by spectrophotometry (US Pharmacopieal Convention, Inc., 1980) and polarography (Mishra & Gode, 1985). The separation and identification of nitrofural in medicated feeds have been reviewed by Fishbein (1972). High-performance liquid chromatography methods for analysing nitrofural in medicated feeds have also been reported (Cieri, 1979; Thorpe, 1980).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Groups of 50 male and 50 female B6C3F_1 mice, seven to eight weeks of age, were administered nitrofural (99% pure) at 0, 150 or 310 mg/kg of diet for 103 weeks; all surviving animals were killed at 112 weeks. The average amount of nitrofural consumed per day was 14-16 mg/kg bw for low-dose male and female mice and 29-33 mg/kg bw for high-dose animals. Survival of high-dose males was lower than that of controls after week 88. At the end of the experiment, survival was: 39/50, 31/50 and 27/50 control, low-dose and high-dose males, and 39/50, 40/50 and 35/50 control, low-dose and high-dose females. Ovarian atrophy was found in 7/47

controls, 44/50 low-dose and 38/50 high-dose mice. Granulosa-cell tumours of the ovary developed in 4/50 low-dose and 9/50 high-dose females ($p = 0.03$, incidental tumour test for trend) compared with 1/47 controls. The incidence of benign mixed tumours of the ovary was 17/50 low-dose and 20/50 high-dose animals ($p < 0.001$, incidental tumour test for trend); no such tumour occurred among controls. No significant difference in the incidence of other types of tumour was observed among treated or control mice (National Toxicology Program, 1988; Kari *et al.*, 1989).

Rat: A group of 30 female weanling Sprague-Dawley rats were administered nitrofural (pharmaceutical grade) at 1000 mg/kg of diet for 46 weeks (daily intake, 8-13 mg/rat), after which they were maintained on control diet for 20 weeks. A control group of 30 rats received control diet for 66 weeks. Of the treated females that lived 22 weeks or more, 22/29 developed mammary fibroadenomas, compared with 2/29 controls (Ertürk *et al.*, 1970). [The Working Group noted that data on survival were not given.]

Groups of 50 male and 50 female Fischer 344/N rats, six to seven weeks of age, were administered nitrofural (99% pure) at 0, 310 or 620 mg/kg of diet for 103 weeks. The average amount of nitrofural consumed per day was 11-12 mg/kg bw for low-dose male and female rats and 24-26 mg/kg bw for high-dose animals. All surviving animals were killed at 111 weeks. Survival in high-dose males was lower than that in controls after week 92. At the end of the experiment, survival was: 33/50, 30/50 and 20/50 controls, low-dose and high-dose males, and 28/50, 37/50 and 31/50 controls, low-dose and high-dose females, respectively. Adenomas of the sebaceous glands of the skin were observed in high-dose males only (4/50 high-dose *versus* 0/50 control; $p = 0.067$, incidental tumour test). Mammary fibroadenomas occurred in 8/49 control, 36/50 low-dose ($p < 0.001$, incidental tumour test) and 36/50 high-dose females ($p < 0.001$, incidental tumour test; $p < 0.001$, incidental tumour test for trend); adenocarcinomas were also observed in one control and two high-dose females. Mononuclear-cell leukaemias occurred in 21/50 control males, 23/50 low-dose males and 6/50 high-dose males ($p = 0.04$, life-table test); 15/49 control females, 2/25 low-dose females ($p < 0.001$) and 2/50 high-dose females ($p < 0.001$ life-table test). Testicular interstitial-cell tumours occurred in 45/50 controls, 30/50 low-dose males ($p < 0.001$, incidental tumour test) and 28/50 high-dose males ($p < 0.001$, incidental tumour test; $p < 0.001$, incidental tumour test for trend) (National Toxicology Program, 1988; Kari *et al.*, 1989).

(*b*) *Transplacental administration*

Mouse: A group of 20 pregnant ICR/Jcl mice, 10-12 weeks of age, received three subcutaneous injections of nitrofural [purity unspecified] at 75 μg/g bw suspended in 1% gelatin solution on days 13, 15 and 17 of gestation. Offspring were foster-nursed by untreated dams and were killed 32 weeks after birth. Treatment

with nitrofural resulted in a marked reduction in the number of live births. At 32 weeks, 67/145 treated animals and 548/844 controls were still alive. The incidence of lung tumours was not significantly increased in nitrofural-treated mice as compared with gelatin-treated controls. All tumours reported were papillary adenomas of the lung (Nomura *et al.*, 1984). [The Working Group noted the short duration and limited reporting of the experiment; interlitter variation was not recorded.]

A group of newborn ICR/Jcl mice, exposed transplacentally as described above, received a subcutaneous injection of nitrofural [purity unspecified] at 75 μg/g bw suspended in 1% gelatin solution within 12 h of birth; three further injections were given on days 7, 14 and 21 after birth. A further group of mice received treatment with gelatin only, and another received no treatment. At 32 weeks, 61/176 treated animals and 548/844 controls were still alive. The number of tumour-bearing mice was 12/61 (19.7%; $p < 0.001$, χ^2 test with Yates' correction against gelatin controls) compared to 5/203 (2.5%) untreated controls. All tumours reported were papillary adenomas of the lung (Nomura *et al.*, 1984). [The Working Group noted the short duration and limited reporting of the experiment; interlitter variation was not recorded.]

3.2 Other relevant data

(*a*) *Experimental systems*

(i) *Absorption, distribution, excretion and metabolism*

Within 24 h after a single oral administration of 100 mg/kg bw ^{14}C-nitrofural to rats, about two-thirds of the radioactivity appeared in the urine, 26% in the faeces and approximately 1% in expired carbon dioxide; complete recovery of the administered dose was observed after 96 h, less than 15% of the label being recovered as unchanged parent compound (Tatsumi *et al.*, 1971). Major metabolites of nitrofural detected and identified in the urine of dosed rats included hydroxylaminofuraldehyde semicarbazone, aminofuraldehyde semicarbazone and 4-cyano-2-oxobutyraldehyde semicarbazone (Paul *et al.*, 1960). The reduced nitrofural metabolite, 4-cyano-2-oxobutyraldehyde semicarbazone, was detected in the urine of germ-free rats treated with the drug (Yeung *et al.*, 1983). Binding of ^{14}C label to liver protein, DNA, ribosomal RNA and kidney protein was demonstrated in rats after oral administration of ^{14}C-nitrofural (Tatsumi *et al.*, 1977).

Nitrofural is reduced by mouse liver homogenate and by several mammalian cell lines, most efficiently under gas mixtures containing 5% O_2 or less (Paul *et al.*, 1960; Olive & McCalla, 1975)

(ii) *Toxic effects*

Oral LD_{50} values of 590 mg/kg bw in rats and 460 mg/kg bw in mice have been reported (Miyaji, 1971). Mice and rats receiving 300 mg/kg bw or more orally showed hyperirritability, tremors and seizures and died from respiratory arrest (Krantz & Evans, 1945). In mice and rats, subcutaneous injection of large doses (3 g/kg bw) produced marked changes in the structure of the liver and kidney, but only slight hepatic changes were seen after lethal oral doses (45 mg/kg bw for four to six days) (Dodd, 1946).

Toxicity was studied by feeding diets containing nitrofural (99% pure) to groups of F344/N rats and $B6C3F_1$ mice for 14 days, 13 weeks or two years. In the 14-day studies, in which the doses ranged from 630 to 10 000 mg/kg of diet, nitrofural was more toxic to mice than to rats. In the 13-week studies, doses for rats ranged from 150 to 2500 mg/kg of diet and for mice from 70 to 1250 mg/kg of diet. At the higher doses, convulsive seizures and gonadal hypoplasia were observed in both species. Evidence of toxicity in rats also included degenerative arthropathy. In the two-year studies (see section 3.1), nitrofural caused testicular degeneration (atrophy of germinal epithelium and aspermatogenesis) in rats and degeneration of vertebral and knee articular cartilage in rats of each sex. In mice of each sex, nitrofural administration induced stimulus-sensitive convulsive seizures, primarily during the first year of study (National Toxicology Program, 1988; Kari *et al.*, 1989).

(iii) *Effects on reproduction and prenatal toxicity*

The gonadotoxicity of nitrofural in male mice has been recognized for more than three decades. Nissim (1957) showed that administration to mice in the diet at a dose equivalent to 375 mg/kg bw caused testicular atrophy. Similar degeneration was observed in rat testis following daily doses of 100 mg/kg bw by gastric intubation for seven days (Miyaji *et al.*, 1964).

In male Sprague-Dawley rats given nitrofural in the diet at a dosage equivalent to 64 mg/kg bw per day for 28 days, the mean weight of the testes was only 28% that of the controls. All stages of spermatogenesis were affected, but Sertoli cells and Leydig cells were not damaged (Hagenäs *et al.*, 1978).

After a single subcutaneous injection to ICR/Jcl mice of nitrofural at 300 mg/kg bw on day 10 of gestation, increased embryo- and fetomortality and decreased fetal weight were observed compared with controls. A significant ($p < 0.001$) increase in the incidence of malformations was observed, predominantly affecting the limbs, digits and tail. After administration of nitrofural at 100 mg/kg bw subcutaneously on days 9-11, the only significant effect observed was a reduction in fetal weight (Nomura *et al.*, 1984).

Pregnant CD1 mice were given nitrofural in the diet at doses equivalent to 6.3-82 mg/kg bw from days 6-15 of gestation. No teratogenic effect was observed,

but there was increased fetal death and reduced fetal weight at the highest dose (National Toxicology Program, 1988).

(iv) *Genetic and related effects*

The genetic toxicology of nitrofurans has been reviewed (Klemencic & Wang, 1978; McCalla, 1983).

Nitrofural inhibited DNA synthesis (Lu & McCalla, 1978) and caused prophage induction in *Escherichia coli* (McCalla & Voutsinos, 1974). It induced DNA strand breaks in *E. coli* (McCalla *et al.*, 1971; Tu & McCalla, 1975; Wentzell & McCalla, 1980) and in *Salmonella typhimurium* strain TA1975 (McCalla *et al.*, 1975). Nitrofural induced differential toxicity in *E. coli* (Yahagi *et al.*, 1974; Haveland-Smith *et al.*, 1979; Lu *et al.*, 1979) and *Bacillus subtilis* (Tanooka, 1977) but not *S. typhimurium* (Yahagi *et al.*, 1974).

Nitrofural induced mutations in *E. coli* (Zampieri & Greenberg, 1964; McCalla & Voutsinos, 1974; Yahagi *et al.*, 1974; McCalla *et al.*, 1975; Tanooka, 1977; Haveland-Smith *et al.*, 1979; Lu *et al.*, 1979; Ebringer & Bencova, 1980; Clarke & Shankel, 1989) in the absence of an exogenous metabolic system, but not in strains lacking nitroreductase activity (McCalla & Voutsinos, 1974; McCalla *et al.*, 1975). In the presence of a microsomal preparation from *Drosophila melanogaster*, nitrofural induced mutations in *E. coli* (Baars *et al.*, 1980). It was not mutagenic to *S. typhimurium* strains TA1535, TA1536, TA1537 or TA1538 (Yahagi *et al.*, 1974; McCalla *et al.*, 1975) but induced mutations in TA1535 in a fluctuation test (Green *et al.*, 1977) and in plate incorporation tests, only in the presence of an exogenous metabolic system (Zeiger *et al.*, 1987; National Toxicology Program, 1988). Nitrofural induced mutations in *S. typhimurium* TA100 and in TA98 in the presence and absence of an exogenous metabolic system (Yahagi *et al.*, 1976; Goodman *et al.*, 1977; Green *et al.*, 1977; Chin *et al.*, 1978; Rosin & Stich, 1978; Bruce & Heddle, 1979; Haveland-Smith *et al.*, 1979; Imamura *et al.*, 1983; Obaseiki-Ebor & Akerele, 1986; Ni *et al.*, 1987; Zeiger *et al.*, 1987; National Toxicology Program, 1988).

Nitrofural was mutagenic to *Neurospora crassa* (Ong, 1977) but not to *Aspergillus nidulans* (Bignami *et al.*, 1982).

Feeding of *Drosophila melanogaster* for three days with nitrofural at 5 mM did not induce sex-linked recessive lethal mutations (Kramers, 1982).

Nitrofural inhibited DNA synthesis in mouse L-929 cells (Olive, 1979a,b). It induced DNA strand breaks in human KB, Syrian hamster BHK-21 and mouse L-929 cells (Olive & McCalla, 1975; Olive, 1978). No unscheduled DNA synthesis was induced by nitrofural in either rat or mouse primary hepatocytes (Mori *et al.*, 1987) or in human fibroblasts (Tonomura & Sasaki, 1973).

Nitrofural induced mutations to 6-thioguanine resistance in Chinese hamster lung (V79) cells (Olive, 1981) but not in Chinese hamster ovary (CHO) cells

(Anderson & Phillips, 1985), either in the presence or absence of an exogenous metabolic system. It induced mutations at the *tk* locus of mouse L5178Y lymphoma cells (National Toxicology Program, 1988). Nitrofural induced sister chromatid exchange in CHO cells in the presence and absence of an exogenous metabolic system (National Toxicology Program, 1988). It induced chromosomal aberrations in Chinese hamster lung cells in the presence and absence of an exogenous metabolic system (Matsuoka *et al.*, 1979; Ishidate, 1988). In CHO cells, however, nitrofural induced chromosomal aberrations in the absence, but not in the presence, of an exogenous metabolic system (National Toxicology Program, 1988). Nitrofural did not induce chromosomal aberrations in human lymphocytes *in vitro* (Tonomura & Sasaki, 1973).

It was not active in micronucleus tests either in rats treated twice with 7.5-30 mg/kg bw intraperitoneally at 30 h and 6 h before they were killed (Goodman *et al.*, 1977) or in mice treated with 150 mg/kg bw intraperitoneally on five consecutive days (Bruce & Heddle, 1979). Nitrofural did not induce chromosomal aberrations in bone-marrow cells of rats either after a single intraperitoneal injection of 60 mg/kg bw (Goodman *et al.*, 1977) or after single oral doses of 40-400 mg/kg bw or five daily oral doses of 15-150 mg/kg bw (Anderson & Phillips, 1985).

Nitrofural did not induce sperm abnormalities in mice treated intraperitoneally with 15-150 mg/kg bw on five consecutive days (Bruce & Heddle, 1979).

(*b*) *Humans*

(i) *Pharmacokinetics*

Nitrofural is not significantly absorbed from skin or mucous membranes after local administration (Marion-Landais *et al.*, 1975; Harvey, 1985).

(ii) *Adverse effects*

Sensitization and generalized allergic skin reactions are known adverse effects of topically administered nitrofural. In a literature review of studies published in 1945-65, 176 (1.2%) cases of skin reactions were reported among 15 162 treated patients (Glascock *et al.*, 1969; Reynolds, 1989).

Nausea, vomiting, joint pains, headaches and polyneuritis are typical toxic effects after oral administration (Reynolds, 1989). Polyneuropathy is common among trypanosomiasis patients treated with nitrofural (Cancado *et al.*, 1964; Robertson & Knight, 1964; Spencer *et al.*, 1975).

Nitrofural has been reported to cause haemolytic anaemia in individuals with glucose-6-phosphate dehydrogenase deficiency (see Prankerd, 1962).

(iii) *Effects on reproduction and prenatal toxicity*

In the Collaborative Perinatal Project, in which drug intake and pregnancy outcome were studied in a series of 50 282 women in 1959-65, 234 women had been

exposed to nitrofural administered topically during the first trimester of pregnancy. Fifteen malformed children were born in the exposed group, giving a standardized relative risk of 0.99 (Heinonen *et al.*, 1977).

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

In a hypothesis-generating cohort study designed to screen a large number of drugs for possible carcinogenicity (described in detail in the monograph on ampicillin), 317 persons to whom at least one prescription for nitrofural had been dispensed during 1969-73 were followed up for up to 15 years (Selby *et al.*, 1989). No statistically significant association was noted with cancer at any site or at all sites combined. [The Working Group noted that the number of users was small and therefore the power of the study to detect carcinogenic effects was probably low. Data on duration of use were not provided.]

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Nitrofural is an antibacterial agent used since 1945 mainly for the local treatment of skin infections. It has been used orally in the treatment of refractory African trypanosomiasis.

4.2 Experimental carcinogenicity data

Nitrofural was tested by oral administration in one study in mice and in two studies in rats, and by transplacental administration to mice. Oral administration to mice increased the incidence of granulosa-cell and benign mixed tumours of the ovary. In rats, an increased incidence of mammary fibroadenomas was observed in females in both studies. Two studies of transplacental administration of nitrofural to mice were inadequate for evaluation.

4.3 Human carcinogenicity data

In a hypothesis-generating cohort study, use of nitrofural was not associated with an increase in cancer incidence, but the power of the study was low.

4.4 Other relevant data

One study did not provide evidence that topical use of nitrofural during pregnancy is associated with birth defects. Nitrofural is gonadotoxic in male and female mice and in male rats and is teratogenic in mice.

In humans, nitrofural is poorly absorbed from skin and mucous membranes after local administration. The drug binds to liver protein and DNA as well as to kidney protein in rats treated *in vivo*.

Nitrofural did not induce chromosomal aberrations in rats, micronuclei in mice or rats or sperm abnormalities in mice. It induced sister chromatid exchange in Chinese hamster cells *in vitro*; contradictory results were obtained on the induction of chromosomal aberrations in mammalian cells. Nitrofural induced DNA strand breaks in human, hamster and mouse cells but did not induce unscheduled DNA synthesis in human, rat or mouse cells. Both positive and negative results were obtained in gene mutation assays in rodent cells. Nitrofural did not induce sex-linked recessive lethal mutations in *Drosophila*. It was mutagenic to *Neurospora* but not to *Aspergillus* and induced differential toxicity in *Escherichia coli* and *Bacillus subtilis* and mutations in *E. coli* and *Salmonella typhimurium*. (See Appendix 1.)

4.5 Evaluation[1]

There is *inadequate evidence* for the carcinogenicity of nitrofural in humans.

There is *limited evidence* for the carcinogenicity of nitrofural in experimental animals.

Overall evaluation

Nitrofural *is not classifiable as to its carcinogenicity to humans (Group 3)*.

5. References

Anderson, D. & Phillips, B.J. (1985) Nitrofurazone—genotoxicity studies in mammalian cells *in vitro* and *in vivo*. *Food. chem. Toxicol.*, *23*, 1091-1098

Anon. (1979) *Pharmaceutical Codex*, 11th ed., London, The Pharmaceutical Press, p. 598

Baars, A.J., Blijleven, W.G.H., Mohn, G.R., Natarajan, A.T. & Breimer, D.D. (1980) Preliminary studies on the ability of *Drosophila* microsomal preparations to activate mutagens and carcinogens. *Mutat. Res.*, *72*, 257-264

Barnhart, E. (1989) *Physicians' Desk Reference*, 43rd ed., Oradell, NJ, Medical Economics, p. 320

Bignami, M., Carere, A., Conti, G., Conti, L., Crebelli, R. & Fabrizi, M. (1982) Evaluation of 2 different genetic markers for the detection of frameshift and missense mutagens in *A. nidulans*. *Mutat. Res.*, *97*, 293-302

[1]For description of the italicized terms, see Preamble, pp. 26–29.

Bruce, W.R. & Heddle, J.A. (1979) The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. *Can. J. genet. Cytol.*, *21*, 319-334

Cancado, J.R., Marra, U.D. & Brener, Z. (1964) Clinical trial of 5-nitro-2-furaldehyde-semicarbazone (nitrofurazone) in chronic form of Chagas's disease. *Rev. Inst. Med. trop. Sao Paulo*, *6*, 12-16 [*Trop. Dis. Bull.*, *61*, 608]

Chamberlain, R.E. (1976) Chemotherapeutic properties of prominent nitrofurans. *J. antimicrob. Chemother.*, *2*, 325-332

Chemical Information Services (1989-90) *Directory of World Chemical Producers 1989/90*, Oceanside, NY

Chin, J.B., Sheinin, D.M.K. & Rauth, A.M. (1978) Screening for the mutagenicity of nitro-group containing hypoxic cell radiosensitizers using *Salmonella typhimurium* strains TA100 and TA98. *Mutat. Res.*, *58*, 1-10

Cieri, U.R. (1979) High pressure liquid chromatographic detection and estimation of furazolidone and nitrofurazone in animal feeds. *J. Assoc. off. anal. Chem.*, *62*, 168-170

Clarke, C.H. & Shankel, D.M. (1989) Antimutagenic specificity against spontaneous and nitrofurazone-induced mutations in *Escherichia coli* K12ND160. *Mutagenesis*, *4*, 31-34

Dodd, M.C. (1946) The chemotherapeutic properties of 5-nitro-2-furaldehyde semicarbazone (Furacin). *J. Pharmacol. exp. Ther.*, *86*, 311-323

Dodd, M.C. & Stillman, W.B. (1944) The *in vitro* bacteriostatic action of some simple furan derivatives. *J. Pharmacol. exp. Ther.*, *82*, 11-18

Ebringer, L. & Bencova, M. (1980) Mutagenicity of nitrofuran drugs in bacterial systems. *Folia microbiol.*, *25*, 388-396

Ertürk, E., Morris, J.E., Cohen, S.M., Price, J.M. & Bryan, G.T. (1970) Transplantable rat mammary tumors induced by 5-nitro-2-furaldehyde semicarbazone and by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide. *Cancer Res.*, *30*, 1409-1412

Fishbein, L. (1972) *Chromatography of Environmental Hazards*, Vol. 1, *Carcinogens, Mutagens and Teratogens*, Amsterdam, Elsevier

Gever, G. & O'Keefe, C.J. (1960) Azomethines of 5-nitro-2-formylfuran with hydrazine compounds. US Patent 2 927 110, 1 March

Glascock, H.W., Jr, MacLeod, P.F., Davis, J.B., Cuddihy, R.V. & Anzlowar, B.R. (1969) Is nitrofurazone a primary irritant or a potent sensitizer? A review of the literature, 1945-1965, and cases reported to the Medical Director. *Rev. Allergy appl. Immunol.*, *23*, 52-58

Goodman, D.R., Hakkinen, P.J., Nemenzo, J.H. & Vore, M. (1977) Mutagenic evaluation of nitrofuran derivatives in *Salmonella typhimurium*, by the micronucleus test, and by in vivo cytogenetics. *Mutat. Res.*, *48*, 295-306

Green, M.H.L., Rogers, A.M., Muriel, W.J., Ward, A.C., & McCalla, D.R. (1977) Use of a simplified fluctuation test to detect and characterize mutagenesis by nitrofurans. *Mutat. Res.*, *44*, 139-143

Hagenäs, L., Plöen, L. & Ritzen, E.M. (1978) The effect of nitrofurazone on the endocrine, secretory and spermatogenic functions of the rat testis. *Andrologia*, *10*, 107-126

Harvey, S.C. (1985) Antiseptics and disinfectants; fungicides; ectoparasiticides. In: Gilman, A., Goodman, L.S., Rall, T.W. & Murad, F., eds, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 7th ed., New York, MacMillan, pp. 967-968

Haveland-Smith, R.B., Combes, R.D. & Bridges, B.A. (1979) Methodology for the testing of food dyes for genotoxic activity: experiments with Red 2G (C.I. 18050). *Mutat. Res.*, *64*, 241-248

Heinonen, O.P., Slone, D. & Shapiro, S. (1977) *Birth Defects and Drugs in Pregnancy*, Littleton, MA, Publishing Sciences Group, pp. 296-313

IARC (1974) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 7, *Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals*, Lyon, pp. 171-180

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating* of IARC Monographs *Volumes 1 to 42*, Lyon, p. 71

Imamura, A., Kurumi, Y., Danzuka, T., Kodama, M., Kawachi, T. & Nagao, M. (1983) Classification of compounds by cluster analysis of Ames test data. *Gann*, *74*, 196-204

Ishidate, M., Jr, ed. (1988) *Data Book of Chromosomal Aberration Test* In Vitro, Amsterdam, Elsevier

Kari, F.W., Huff, J.E., Leininger, J., Haseman, J.K. & Eustis, S.L. (1989) Toxicity and carcinogenicity of nitrofurazone in F344/N rats and B6C3F$_1$ mice. *Chem. Toxicol.*, *27*, 129-137

Klemencic, J.M. & Wang, C.Y. (1978) Mutagenicity of nitrofurans. In: Bryan, G.T., ed., *Carcinogenesis*, Vol. 4, *Nitrofurans*, New York, Raven Press, pp. 99-130

Kramers, P.G.N. (1982) Studies on the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* by nitroheterocyclic compounds. *Mutat. Res.*, *101*, 209-236

Krantz, J.C. & Evans, W.E. (1945) A contribution to the pharmacology of 5-nitro-2-furaldehyde semicarbazone. *J. Pharmacol. exp. Ther.*, *85*, 324-331

Lu, C. & McCalla, D.R. (1978) Action of some nitrofuran derivatives on glucose metabolism, ATP levels, and macromolecule synthesis in *Escherichia coli*. *Can. J. Microbiol.*, *24*, 650-657

Lu, C., McCalla, D.R. & Bryant, D.W. (1979) Action of nitrofurans on *E. coli*. Mutation and induction and repair of daughter-strand gaps in DNA. *Mutat. Res.*, *67*, 133-144

Marion-Landais, G., Heatis, J.P. & Herrett, R.J. (1975) Non-absorption of furazolidone from the vagina in women. *Curr. Ther. Res.*, *18*, 510-512

Matsuoka, A., Hayashi, M. & Ishidate, M., Jr (1979) Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. *Mutat. Res.*, *66*, 277-290

McCalla, D.R. (1983) Mutagenicity of nitrofuran derivatives: review. *Environ. Mutagenesis*, *5*, 745-765

McCalla, D.R. & Voutsinos, D. (1974) On the mutagenicity of nitrofurans. *Mutat. Res.*, *26*, 3-16

McCalla, D.R., Reuvers, A. & Kaiser, C. (1971) Breakage of bacterial DNA by nitrofuran derivatives. *Cancer Res.*, *31*, 2184-2188

McCalla, D.R., Voutsinos, D. & Olive, P.L. (1975) Mutagen screening with bacteria: niridazole and nitrofurans. *Mutat. Res.*, *31*, 31-37

Mishra, A.K. & Gode, K.D. (1985) Electrochemical reduction of nitrofurazone and its determination in pharmaceutical dosage forms by d.c. polarography. *Analyst*, *110*, 1373-1376

Miura, K. & Reckendorf, H.K. (1967) The nitrofurans. In: Ellis, G.P. & West, G.B., eds, *Progress in Medicinal Chemistry*, Vol. 5, New York, Plenum, pp. 320-381

Miyaji, T. (1971) Acute and chronic toxicity of furylfuramide in rats and mice. *Tohoku J. exp. Med.*, *103*, 331-369

Miyaji, T., Miyamoto, M. & Ueda, Y. (1964) Inhibition of spermatogenesis and atrophy of the testis caused by nitrofuran compounds. *Acta pathol. jpn.*, *14*, 261-273

Mori, H., Sugie, S., Yoshimi, N., Kinouchi, T. & Ohnishi, Y. (1987) Genotoxicity of a variety of nitroarenes and other nitro compounds in DNA-repair tests with rat and mouse hepatocytes. *Mutat. Res.*, *190*, 159-167

Morris, J.E., Price, J.M., Lalich, J.J. & Stein, R.J. (1969) The carcinogenic activity of some 5-nitrofuran derivatives in the rat. *Cancer Res.*, *29*, 2145-2156

National Toxicology Program (1988) *Toxicology and Carcinogenesis Studies of Nitrofurazone (CAS No. 59-87-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)* (NTP Technical Report 337), Research Triangle Park, NC, pp. 18-19, 160-164

Ni, Y.-C., Heflich, R.H., Kadlubar, F.F. & Fu, P.P. (1987) Mutagenicity of nitrofurans in *Salmonella typhimurium* TA98, TA98NR and TA98/1,8-DNP$_6$. *Mutat. Res.*, *192*, 15-22

Nissim, J. A. (1957) Increased pituitary gonadotrophin activity after degeneration of seminiferous tubules produced by nitrofurazone. *Lancet*, *i*, 304-305

Nomura, T., Kimura, S., Kanzaki, T., Tanaka, H., Shibata, K., Nakajima, H., Isa, T., Kurokawa, N., Hatanaka, T., Kinuta, M., Masada, K. & Sakamoto, Y. (1984) Induction of tumors and malformations in mice after prenatal treatment with some antibiotic drugs. *Med. J. Osaka Univ.*, *35*, 13-17

Obaseiki-Ebor, E.E. & Akerele, J.O. (1986) Nitrofuran mutagenicity: induction of frameshift mutations. *Mutat. Res.*, *175*, 149-152

Olive, P.L. (1978) Nitrofurazone-induced DNA damage to tissues of mice. *Chem.-biol. Interactions*, *20*, 323-331

Olive, P.L. (1979a) Inhibition of DNA synthesis by nitroheterocycles I. Correlation with half-wave reduction potential. *Br. J. Cancer*, *40*, 89-93

Olive, P.L. (1979b) Inhibition of DNA synthesis by nitroheterocycles II. Mechanisms of cytotoxicity. *Br. J. Cancer*, *40*, 94-104

Olive, P.L. (1981) Correlation between the half-wave reduction potentials of nitroheterocycles and their mutagenicity in Chinese hamster V79 spheroids. *Mutat. Res.*, *82*, 137-145

Olive, P.L. & McCalla, D.R. (1975) Damage to mammalian cell DNA by nitrofurans. *Cancer Res.*, *35*, 781-784

Ong, T.-M. (1977) Mutagenic activities of nitrofurans in *Neurospora crassa*. *Mutat. Res.*, *56*, 13-20

Paul, H.E., Ells, V.R., Kopko, F. & Bender, R.C. (1960) Metabolic degradation of the nitrofurans. *J. med. pharm. Chem.*, *2*, 563-584

Prankerd, T.A.J. (1962) Hemolytic effects of drugs and chemical agents. *Clin. Pharmacol. Ther.*, *4*, 334-350

Reynolds, J.E.F., ed. (1982) *Martindale. The Extra Pharmacopoeia*, 28th ed., London, The Pharmaceutical Press, pp. 499-500

Reynolds, J.E.F., ed. (1989) *Martindale. The Extra Pharmacopoeia*, 29th ed., London, The Pharmaceutical Press, p. 674

Robertson, D.H.H. & Knight, R.H. (1964) Observations on the polyneuropathy and the disordered pyruvate metabolism induced by nitrofurazone in cases of sleeping sickness due to *Trypanosoma rhodesiense*. *Acta trop.*, *21*, 239-263 [*Trop. Dis. Bull.*, *62*, 179]

Rosin, M.P. & Stich, H.F. (1978) The inhibitory effect of cysteine on the mutagenic activities of several carcinogens. *Mutat. Res.*, *54*, 73-81

Selby, J.V., Friedman, G.D. & Fireman, B.H. (1989) Screening prescription drugs for possible carcinogenicity: 11 to 15 years of follow-up. *Cancer Res.*, *49*, 5736-5747

Spencer, H.C., Jr, Gibson, J.J., Jr, Brodsky, R.E. & Schultz, M.G. (1975) Imported African trypanosomiasis in the United States. *Ann. intern. Med.*, *82*, 633-638

Stillman, W.B. & Scott, A.B. (1947) Substituted nitrofurans. US Patent 2 416 234, 18 February

Tanooka, H. (1977) Development and applications of *Bacillus subtilis* test systems for mutagens, involving DNA-repair deficiency and suppressible auxotrophic mutations. *Mutat. Res.*, *42*, 19-32

Tatsumi, K., Ou, T., Yoshimura, H. & Tsukamoto, H. (1971) Metabolism of drugs. LXXIII. The metabolic fate of nitrofuran derivatives. (3) Studies on enzymes in small intestinal mucosa of rat catalyzing degradation of nitrofuran derivatives. *Chem. pharm. Bull.*, *21*, 622-628

Tatsumi, K., Kitamura, S. & Yoshimura, H. (1977) Binding of nitrofuran derivatives to nucleic acids and protein. *Chem. pharm. Bull.*, *25*, 2948-2952

Thorpe, V.A. (1980) Sample preparation of carbadox, furazolidone and ethopabate in medicated feeds for high pressure liquid chromatography. *J. Assoc. off. anal. Chem.*, *63*, 981-984

Tonomura, A. & Sasaki, M.S. (1973) Chromosome aberrations and DNA repair synthesis in cultured human cells exposed to nitrofurans. *Jpn. J. Genet.*, *48*, 291-294

Tu, Y. & McCalla, D.R. (1975) Effect of activated nitrofurans on DNA. *Biochim. biophys. Acta*, *402*, 142-149

US Pharmacopeial Convention, Inc. (1980) *US Pharmacopeia*, 20th rev., Rockville, MD, pp. 550-551

US Tariff Commission (1956) *Synthetic Organic Chemicals, US Production and Sales 1955* (Second Series, Report No. 198), Washington DC, US Government Printing Office, p. 112

Wentzell, B. & McCalla, D.R. (1980) Formation and excision of nitrofuran-DNA adducts in *Escherichia coli*. *Chem.-biol. Interactions*, *31*, 133-150

Windholz, M., ed. (1983) *The Merck Index*, 10th ed., Rahway, NJ, Merck & Co., p. 947

Yahagi, T., Nagao, M., Hara, K., Matsushima, T., Sugimura, T. & Bryan, G.T. (1974) Relationships between the carcinogenic and mutagenic or DNA-modifying effects of nitrofuran derivatives, including 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, a food additive. *Cancer Res.*, *34*, 2266-2273

Yahagi, T., Matsushima, T., Nagao, M., Seino, Y., Sugimura, T. & Bryan, G.T. (1976) Mutagenicities of nitrofuran derivatives on a bacterial tester strain with an R factor plasmid. *Mutat. Res.*, *40*, 9-14

Yeung, T.C., Sudlow, G., Koch, R.L. & Goldman, P. (1983) Reduction of nitroheterocyclic compounds by mammalian tissues in vivo. *Biochem. Pharmacol.*, *32*, 2259-2263

Zampieri, A. & Greenberg, J. (1964) Nitrofurazone as a mutagen in *Escherichia coli*. *Biochem. biophys. Res. Commun.*, *14*, 172-176

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. & Speck, W. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagenesis*, *9* (*Suppl. 9*), 1-110