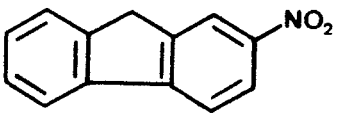
2-NITROFLUORENE

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 607-57-8
Chem. Abstr. Name: 9*H*-Fluorene, 2-nitro-
IUPAC Systematic Names: 2-Nitro-9*H*-fluorene; 2-nitrofluorene

1.2 Structural and molecular formulae and molecular weight



$C_{13}H_9NO_2$ Mol. wt: 211.2

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Needles, recrystallized from 50% acetic acid or acetone (Weast, 1985); light-yellow, fluffy solid (Chemsyn Science Laboratories, 1988)

(*b*) *Melting-point*: 156°C (Buckingham, 1982)

(*c*) *Spectroscopy data*: Ultra-violet (Sawicki, 1954) and mass (Schuetzle & Jensen, 1985) spectral data have been reported.

(*d*) *Solubility*: Sparingly soluble in water (Beije & Möller, 1988); soluble in acetone, benzene (Weast, 1985), tetrahydrofluorenone and toluene (Chemsyn Science Laboratories, 1988)

1.4 Technical products and impurities

2-Nitrofluorene is available for research purposes at 95% (Pfaltz & Bauer, Inc. 1988), 98% (Aldrich Chemical Co., 1988) or ≥99% purity and in radiolabelled form at ≥98% [^{14}C] or ≥99% [^{3}H] purity (Chemsyn Science Laboratories, 1988).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

No evidence was found that 2-nitrofluorene is currently produced other than for laboratory use. It is reported on the 1985 *Toxic Substances Control Act Chemical Substance Inventory* (US Environmental Protection Agency, 1986).

(*b*) *Use*

No evidence was found that 2-nitrofluorene has been used for commercial applications.

2.2 Occurrence

(*a*) *Engine exhaust*

2-Nitrofluorene has been identified in diesel emissions. Levels of two isomers of nitrofluorene in exhausts from light-duty diesel passenger cars were 71–186 mg/kg of particulates (Schuetzle, 1983). Emission levels from 1980–85 model engines running on an urban Federal Test Procedure cycle were 90 μg/mile (56 μg/km) for the gas phase and 97 μg/mile (61 μg/km) for the particulate phase (Schuetzle & Frazier, 1986). Concentrations of 0.63 mg/kg in particulates were reported for a heavy-duty mining diesel engine run at 100% load and 1200 revolutions/min and 8.8 mg/kg in particulates for the same engine at 75% load and 1800 revolutions/min (Draper, 1986).

(*b*) *Other occurrence*

Toners for use in photocopy machines have been produced in quantity since the late 1950s and have seen widespread use. 'Long-flow' furnace black was first used in photocopy toners in 1967; its manufacture involved an oxidation whereby some nitration also occurred. Subsequent changes in the production technique reduced the total extractable nitropyrene content from an uncontrolled level of 5–100 mg/kg to below 0.3 mg/kg (Rosenkranz *et al.*, 1980; Sanders, 1981; Butler *et al.*, 1983), and toners produced from this carbon black since 1980 have not been found to contain detectable levels of mutagenicity or, hence, nitropyrenes (Rosenkranz *et al.*, 1980; Butler *et al.*, 1983).

2-Nitrofluorene has been detected in airborne particulates in Tokyo, Japan, at concentrations of 0–21.8 mg/kg and in air at concentrations of 0–27.2 pg/m^3 (Tanabe *et al.*,

1986) and 24–71 pg/m³, in China at 36–700 pg/m³ and in the Federal Republic of Germany at 170–5200 pg/m³ (Möller, 1988; Beije & Möller, 1988).

2-Nitrofluorene was identified in particulate extracts of emissions from kerosene heaters, gas burners and liquefied petroleum gas burners (Tokiwa *et al.*, 1985). In the exhaust from an open, oil-burning space heater, of the type used extensively in Japan in urban and rural residential and public office spaces, a concentration of 568 ng/m³ 2-nitrofluorene was reported (Möller, 1988).

2-Nitrofluorene was found in a river sediment at 1.5 μg/kg (Möller, 1988).

2.3 Analysis

See the monograph on 1-nitropyrene.

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

3.1 Carcinogenicity studies in animals[1]

(a) Oral administration

Rat: A group of six male and three female Minnesota strain [albino] rats [age unspecified] was fed a basal diet containing 2.37 mmol[500 mg]/kg diet 2-nitrofluorene ([purity unspecified] melting-point, 157°C) for 23 weeks (average estimated total dose, 756 mg per rat), after which time animals were placed on basal control diet until they developed gross tumours or became moribund (Morris *et al.*, 1950). Average survival time was 308 days for tumour-bearing animals and 310 days for animals without tumours. Three males and three females fed basal diet alone served as controls (average survival time, 280 days). At the end of the experiment, two females in the treated group had one adenocarcinoma of the mammary gland and one squamous-cell carcinoma of the ear duct; no tumour was observed in controls. [The Working Group noted the small number of animals used.]

Nine male and nine female Holtzman [albino] rats weighing 190–210 g [age unspecified] were fed a basal grain diet containing 1.62 mmol[342 mg]/kg diet 2-nitrofluorene ([purity unspecified] melting-point, 155–157°C) for eight months (Miller *et al.*, 1955). A group of 26 males and 27 females receiving a diet containing 1.62 mmol[361 mg]/kg diet 2-acetylaminofluorene (a metabolite of 2-nitrofluorene) served as positive controls. After eight months, both groups were maintained on grain diet alone for two months. A group of 18 male and 20 female rats received basal grain diet alone for ten months, at which time the experiment was terminated. 2-Acetylaminofluorene induced high incidences of liver-cell tumours in males (24/26) and of mammary gland tumours, described as adenocarcinomas,

[1]The Working Group was aware of a study in progress in mice by single subcutaneous injection (IARC, 1988).

in females (22/25) and caused moderate incidences of carcinomas of the ear duct (11/26 in males, 19/27 in females) and adenocarcinomas of the small intestinal epithelium (13/26 in males, 6/27 in females). Four mammary gland tumours were seen in 2-nitrofluorene-treated females and only one fibroadenoma in an untreated female. Most rats given 2-nitrofluorene developed multiple papillomas or squamous-cell carcinomas in the forestomach (5/7 males and 2/2 females examined). To confirm this observation, a group of 20 male rats was fed 1.62 mmol[342 mg]/kg diet 2-nitrofluorene for 12 months, during which time a further ten males were maintained on basal diet. Of these rats, 17/18 that survived for 10–12 months had squamous-cell carcinomas of the forestomach. In addition, 13 rats in this group had developed tumours of the liver, four had tumours of the ear duct, two had tumours of the small intestinal epithelium, and one had a tumour of the mammary gland by 12 months. No tumour was found in the control group.

(*b*) *Initiation-promotion study*

In a initiation-promotion model, Möller *et al.* (1989) gave weanling male Wistar rats 20, 100 or 200 mg/kg bw 2-nitrofluorene intraperitoneally 16 h after a two-thirds hepatectomy. Two weeks later, the animals were fed a diet supplemented with 0.02% 2-acetylaminofluorene for two weeks, 2 ml/kg bw carbon tetrachloride intragastrically when the rats had been on this diet for one week, and then a basic diet for a further four weeks. In a second experiment, weanling male Wistar rats received an intraperitoneal injection of 200 mg/kg bw *N*-nitrosodiethylamine. Two weeks later, they were given 30 or 120 mg/kg bw 2-nitrofluorene intragastrically in six equal doses: four doses were given on consecutive days followed by a two-thirds hepatectomy, followed by two additional doses of 2-nitrofluorene. Seven weeks after initiation, the rats were killed, and γ-glutamyl transferase-positive liver foci were identified microscopically and quantitified using morphometric techniques. A statistically significant increase ($p < 0.001$) in the number of foci was observed in the first experiment. A dose-response effect was seen in the second study, the highest response being approximately three times the background.

3.2 Other relevant data

(*a*) *Experimental systems*

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

The metabolism of 2-nitrofluorene *in vivo* has been reviewed by Möller (1988).

After male albino rabbits were administered 2-nitrofluorene orally at 100 mg/kg bw per day for two days, 2-aminofluorene, 2-acetylaminofluorene and 2-formylaminofluorene were found in urine collected for three days after dosing. The same metabolites were found in the faeces but not in urine of male Wistar rats treated similarly. 2-Formylaminofluorene was also detected in in-vitro incubations of rabbit, rat, mouse, guinea-pig and hamster cytosol supplemented with *N*-formyl-L-kynurenine and 2-aminofluorene (Tatsumi & Amano, 1987). [The Working Group noted that 2-aminofluorene and 2-acetylamino-

fluorene are carcinogenic in a variety of experimental animal species. See for example, Garner *et al.* (1984).]

In an eight-day period after Sprague-Dawley rats were given a single oral dose of 5 mg [^{14}C]2-nitrofluorene, approximately 60% was excreted in the urine and 30% was found in the faeces; *N*-, 1-, 3-, 5-, 7-, 8- and 9-hydroxy-2-acetylaminofluorenes were identified as metabolites. The major products were 5- and 7-hydroxy-2-acetylaminofluorenes (Möller *et al.*, 1985, 1987a). [The Working Group noted that *N*-hydroxy-2-acetylaminofluorene is carcinogenic in a variety of experimental species and 9-hydroxy-2-acetylaminofluorene in rats. See for example, Garner *et al.*, 1984]. After intratracheal instillation in rats, 2-nitrofluorene was rapidly excreted into the perfusate (Möller *et al.*, 1987b). Intestinal microflora appeared to reduce the excretion of mutagenic metabolites of 2-nitrofluorene (Möller *et al.*, 1988).

As reported in an abstract, covalent binding to haemoglobin was detected in male Sprague-Dawley rats treated orally with 0.5 mmol[106 mg]/kg bw 2-nitrofluorene, although the level was lower than that found with 2-aminofluorene (Suzuki *et al.*, 1987).

Under anaerobic conditions, rat liver microsomes and rabbit liver microsomes and cytosol catalysed the reduction of 2-nitrofluorene to *N*-hydroxy-2-aminofluorene and 2-aminofluorene (Uehleke & Nestel, 1967; Sternson, 1975; Kitamura *et al.*, 1983; Tatsumi *et al.*, 1986). [The Working Group noted that *N*-hydroxy-2-aminofluorene is carcinogenic to rats. See, for example, Garner *et al.*, 1984).]

Incubation of 2-nitrofluorene with postmitochondrial supernatants from the livers of Wistar rats pretreated with sodium phenobarbital, 3-methylcholanthrene or Kenechlor-500 resulted in a time-dependent loss of substrate. The rate of metabolism was faster with homogenates from animals pretreated with 3-methylcholanthrene or Kanechlor-500 than with those from animals given phenobarbital (Ohe, 1985).

The major metabolites in isolated perfused lungs from male Sprague-Dawley rats were 9-hydroxy-2-nitrofluroene and an unidentified hydroxylated nitrofluorene. The same metabolites were detected in perfused livers from Wistar rats following treatment with β-glucuronidase (Möller *et al.*, 1987b).

(ii) *Toxic effects*

No data were available to the Working Group.

(iii) *Genetic and related effects*

The genetic and related effects of nitroarenes and of their metabolites have been reviewed (Rosenkranz & Mermelstein, 1983; Beland *et al.*, 1985; Rosenkranz & Mermelstein, 1985; Tokiwa & Ohnishi, 1986).

2-Nitrofluorene induced DNA damage in *Salmonella typhimurium* (Nakamura *et al.*, 1987; lowest effective dose, 31 μg/ml) and in *Escherichia coli* (Ohta *et al.*, 1984 (50–200 μg/ml); Quillardet *et al.*, 1985; Mamber *et al.*, 1986; Marzin *et al.*, 1986 (lowest effective dose, 100 nM/ml)). Treatment of *E. coli* with 2-nitrofluorene induced binding of cellular DNA to the bacterial envelope (Kubinski *et al.*, 1981). Conflicting results have been reported regarding the ability of 2-nitrofluorene to induce prophage in *E. coli* (Ho & Ho,

1981; Mamber *et al.*, 1984). 2-Nitrofluorene (only dose tested, 10 μg/ml) preferentially inhibited the growth of DNA repair-deficient *E. coli* (Rosenkranz & Poirier, 1979; Doudney *et al.*, 1981; McCarroll *et al.*, 1981a; Mamber *et al.*, 1983) and *Bacillus subtilis* (McCarroll *et al.*, 1981b; Suter & Jaeger, 1982).

Conflicting results have been reported regarding the mutagenicity of 2-nitrofluorene to *E. coli* (Sakamoto *et al.*, 1980; Dunkel *et al.*, 1984; Mitchell & Gilbert, 1984, 1985). Over 200 independent reports are available on the mutagenicity of 2-nitrofluorene in *S. typhimurium*, as the compound is often used as a positive reference compound. These studies gave generally positive results: e.g., mutagenic to *S. typhimurium* TA97, TA98, TA100, TA1538, TA1978 (Purchase *et al.*, 1978; Rosenkranz & Poirier, 1979; Sakamoto *et al.*, 1980; Wang *et al.*, 1980; McCoy *et al.*, 1981; Pederson & Siak, 1981; Tokiwa *et al.*, 1981; Pitts *et al.*, 1982; Rosenkranz & Mermelstein, 1983; Dunkel *et al.*, 1984; Xu *et al.*, 1984; Vance *et al.*, 1987) and BA9 (Ruiz-Rubio *et al.*, 1984; Hera & Pueyo, 1986) but not to strain SV50 (Xu *et al.*, 1984). 2-Nitrofluorene (0.7 μg/ml) was also mutagenic to *Photobacterium leiognathi* (Ulitzur, 1982).

2-Nitrofluorene (5%) induced recombination in the yeast *Saccharomyces cerevisiae* D3 (Simmon, 1979) but not strain D4 (at up to 100 μg/ml; Mitchell, 1980). It was not mutagenic to *Aspergillus nidulans* at up to 2000 μg/ml (Bignami *et al.*, 1982). 2-Nitrofluorene has been reported to be mutagenic to the *Tradescantia* stamen hair (Schairer & Sautkulis, 1982).

In the mouse host-mediated assay, 2-nitrofluorene (at 125–1600 mg/kg) induced mutation in *S. typhimurium* but not recombination in *S. cerevisiae* D3 (Simmon *et al.*, 1979). The urine of rats administered 2-nitrofluorene was mutagenic to *S. typhimurium* (Beije & Möller, 1988).

2-Nitrofluorene caused inhibition of DNA synthesis in HeLa cells (Painter & Howard, 1982). At a dose of 10^{-4}–10^{-1} mg/ml, it induced unscheduled DNA synthesis in mouse hepatocytes (Mori *et al.*, 1987), but conflicting results were obtained with rat hepatocytes: negative at 1000 nmol/ml (Probst *et al.*, 1981) but active at 10^{-4}–10^{-1} mg/ml (Mori *et al.*, 1987). It induced sister chromatid exchange in Chinese hamster CHO cells (at 30 μM) in the presence of an exogenous metabolic system (Nachtman & Wolff, 1982) and mutation in mouse lymphoma L5178Y $TK^{+/-}$ cells (Amacher *et al.*, 1979; Oberly *et al.*, 1984).

2-Nitrofluorene induced morphological transformation of Syrian hamster embryo cells in the presence of hamster hepatocytes (Poiley *et al.*, 1979; Pienta, 1980).

Oral administration of 2-nitrofluorene (125–500 mg/kg) to Chinese hamsters resulted in an increased incidence of sister chromatid exchange in bone-marrow cells; no such effect was observed after intraperitoneal administration of 50–200 mg/kg (Neal & Probst, 1983).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Epidemiological studies and case reports of carcinogenicity in humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

2-Nitrofluorene has been detected in particulate emissions from diesel engines, kerosene heaters and gas burners. It has also been found at low concentrations in ambient air.

4.2 Experimental data

2-Nitrofluorene was tested for carcinogenicity in rats by oral administration, producing tumours of the mammary gland, forestomach, liver and ear duct. In a liver initiation-promotion model, it was shown to be an initiator of preneoplastic foci.

4.3 Human data

No data were available to the Working Group.

4.4 Other relevant data

2-Nitrofluorene induced sister chromatid exchange in Chinese hamsters *in vivo*. It induced DNA damage, sister chromatid exchange, mutation and morphological transformation in cultured animal cells. It was recombinogenic but not mutagenic to fungi and induced DNA damage and mutation in bacteria.

2-Aminofluorene, 2-acetylaminofluorene, *N*-hydroxy-2-amino-fluorene and *N*-hydroxy-2-acetylaminofluorene, which are model carcinogens, have been detected as metabolites of 2-nitrofluorene.

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity in experimental animals of 2-nitrofluorene.

No data were available from studies in humans on the carcinogenicity of 2-nitrofluorene.

Overall evaluation

2-Nitrofluorene is *possibly carcinogenic to humans (Group 2B).*

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[1]For definitions of the italicized terms, see Preamble, pp. 25–28.

Summary table of genetic and related effects of 2-nitrofluorene

Nonmammalian systems													Mammalian systems																									
Prokaryotes		Lower eukaryotes				Plants			Insects				*In vitro*																*In vivo*									
													Animal cells								Human cells								Animals							Humans				
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A
+	+		+	−[1]									+	+	+[1]				+[1]												+[1]									

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the table, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

\+ considered to be positive for the specific endpoint and level of biological complexity

+[1] considered to be positive, but only one valid study was available to the Working Group

−[1] considered to be negative, but only one valid study was available to the Working Group

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