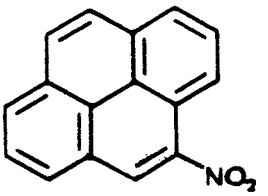
4-NITROPYRENE

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 57835-92-4
Chem. Abstr. Name: Pyrene, 4-nitro-
IUPAC Systematic Name: 4-Nitropyrene

1.2 Structural and molecular formulae and molecular weight



$C_{16}H_9NO_2$ Mol. wt: 247.3

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Slender orange needles (Bavin, 1959)

(*b*) *Melting-point*: 190–192°C (Paputa-Peck *et al.*, 1983); 196–197.5°C (Bavin, 1959)

(*c*) *Spectroscopy data*: Nuclear magnetic resonance and ultra-violet spectral data have been reported (Paputa-Peck *et al.*, 1983).

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

4-Nitropyrene can be produced by the electrophilic nitration of pyrene. No evidence was found that 4-nitropyrene has been produced for other than laboratory use.

(*b*) *Use*

No evidence was found that 4-nitropyrene has been used for commercial applications.

2.2 Occurrence

4-Nitropyrene was found in a sample of ambient airborne particulates collected in Torrance, CA, USA (Korfmacher *et al.*, 1987).

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).

2.3 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

(*a*) *Subcutaneous administration*

Rat: A group of female newborn CD rats [initial numbers unspecified] received subcutaneous injections of 0.1 mmol[25 mg]/kg bw 4-nitropyrene [purity unspecified] dissolved in dimethyl sulfoxide (DMSO) once a week for eight weeks (total dose, 63 μmol [15.6 mg]; King, 1988). A group of rats received DMSO alone. Animals were sacrificed when moribund or at 86 weeks. A statistically significant increase ($p < 0.005$) in the number

of rats with mammary tumours was observed in the treated group (20/27; 18 adenocarcinomas, 14 fibroadenomas; induction period, 263 days) compared with controls (17/47; 16 fibroadenomas; induction period, 502 days). Ten rats developed other malignant tumours (malignant fibrous histiocytomas, leukaemias and ear-duct tumours [numbers unspecified]) that were not observed in controls.

(*b*) *Intraperitoneal administration*

Mouse: Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 4-nitropyrene (total dose, 2800 nmol [692 μg]; purity, >99%) in 10, 20 and 40 μl DMSO on days 1, 8 and 15 of age; a total dose of 560 nmol [140 μg] benzo[*a*]pyrene (purity, >99%); or three injections of DMSO only (Wislocki *et al.*, 1986). Treatment of a second vehicle control group was begun ten weeks after that of the other groups. At 25–27 days, when the mice were weaned, 29 male and 29 female treated mice, 37 male and 27 female positive controls, and 28 and 31 males and 45 and 34 females in the two vehicle control groups were still alive. All remaining mice were killed after one year. Liver-cell tumours developed in 24/29 treated males (four adenomas, 20 carcinomas; $p < 0.005$) and 2/29 treated females (one adenoma and one carcinoma). Male mice also had a higher multiplicity of liver tumours than controls, with an average of 6.0 nodules per tumour-bearing animal. Lung tumours occurred in 11/29 treated males (ten adenomas, one carcinoma; $p < 0.005$) and 9/29 treated females (eight adenomas, one carcinoma; $p < 0.005$). Benzo[*a*]pyrene induced liver-cell tumours in 18/37 males but not in females, and lung adenomas in 13/37 males and 13/27 females ($p < 0.005$). Of the vehicle controls, 2/28 and 5/45 males had liver adenomas and 1/28 and 4/45 had lung tumours, and 0/31 and 0/34 females had liver tumours and 0/31 and 2/34 had lung tumours.

Rat: In a study reported as an abstract, female CD rats [initial number unspecified], 30 days of age, received intraperitoneal injections of 67 μmol[16.5 mg]/kg bw 4-nitropyrene [purity unspecified] three times per week for four weeks (Imaida *et al.*, 1985). Surviving rats were killed at 62 weeks. The incidence of mammary tumours (17/29) was significantly different from that in controls (4/29; $p < 0.001$).

3.2 Other relevant data

(*a*) *Experimental systems*

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

It was reported in an abstract that rat liver microsomes catalysed the conversion of 4-nitropyrene to 4-nitropyrene 9,10-dione, 8-hydroxy-4-nitropyrene and 4-nitropyrene-1,6-hydroquinone (Fu *et al.*, 1986).

(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).

4-Nitropyrene (0.1–2.0 μg/disc) preferentially inhibited the growth of DNA repair-deficient *Bacillus subtilis* (Horikawa *et al.*, 1986; Tokiwa *et al.*, 1987) and was mutagenic to *Salmonella typhimurium* TA98 and TA100 (Fu *et al.*, 1985).

(*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

4-Nitropyrene was detected at a low concentration in ambient air in one study.

4.2 Experimental data

4-Nitropyrene was tested for carcinogenicity in newborn rats by subcutaneous injection, producing an increase in the incidence of mammary tumours. It was also tested by intraperitoneal injection in newborn mice, producing an increase in the incidence of liver-cell tumours in males and of lung tumours in animals of each sex. A study by intraperitoneal injection was inadequately reported.

4.3 Human data

No data were available to the Working Group.

4.4 Other relevant data

4-Nitropyrene induced DNA damage and mutation in bacteria.

Summary table of genetic and related effects of 4-nitropyrene

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]																																						

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

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity in experimental animals of 4-nitropyrene.

No data were available from studies in humans on the carcinogenicity of 4-nitropyrene.

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

5. References

Bavin, P.M.G. (1959) 4-Nitropyrene. *Can. J. Chem., 37*, 1614–1615

Beland, F.A., Heflich, R.H., Howard, P.C. & Fu, P.P. (1985) The in vitro metabolic activation of nitro polycyclic aromatic hydrocarbons. In: Harvey, R.G., ed., *Polycyclic Hydrocarbons and Carcinogenesis (ACS Symposium Series No. 283)*, Washington DC, American Chemical Society, pp. 371–396

Butler, M.A., Evans, D.L., Giammarise, A.T., Kiriazides, D.K., Marsh, D., McCoy, E.C., Mermelstein, R., Murphy, C.B. & Rosenkranz, H.S. (1983) Application of *Salmonella* assay to carbon blacks and toners. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons, 7th International Symposium, Formation, Metabolism and Measurement*, Columbus, OH, Battelle, pp. 225–241

Fu, P.P., Chou, M.W., Miller, D.W., White, G.L., Heflich, R.H. & Beland, F.A. (1985) The orientation of the nitro substituent predicts the direct-acting bacterial mutagenicity of nitrated polycyclic aromatic hydrocarbons. *Mutat. Res., 143*, 173–181

Fu, P.P., Von Tungeln, L.S., Unruh, L.E. & Heflich, R.H. (1986) In vitro metabolism of 4-nitropyrene to a new type of mutagenic metabolite (Abstract No. 157). *Pharmacologist, 28*, 120

Horikawa, K., Sera, N., Tokiwa, H. & Kada, T. (1986) Results of the *rec*-assay of nitropyrenes in the *Bacillus subtilis* test system. *Mutat. Res., 174*, 89–92

Imaida, K., Hirose, M., Lee, M.-S., Wang, C.Y. & King, C.M. (1985) Comparative carcinogenicities of 1-, 2-, and 4-nitropyrenes (NP) and structurally related compounds for female CD rats following intraperitoneal injection (Abstract No. 366). *Proc. Am. Assoc. Cancer Res., 26*, 93

King, C.M. (1988) *Metabolism and Biological Effects of Nitropyrene and Related Compounds (Research Report No. 16)*, Cambridge, MA, Health Effects Institute

Korfmacher, W.A., Rushing, L.G., Arey, J., Zielinska, B. & Pitts, J.N., Jr (1987) Identification of mononitropyrenes and mononitrofluoranthenes in air particulate matter *via* fused silica gas chromatography combined with negative ion atmospheric pressure ionization mass spectrometry. *J. high Resol. Chromatogr. Chromatogr. Commun., 10*, 641–646

Paputa-Peck, M.C., Marano, R.S., Schuetzle, D., Riley, T.L., Hampton, C.V., Prater, T.J., Skewes, L.M., Jensen, T.E., Ruehle, P.H., Bosch, L.C. & Duncan, W.P. (1983) Determination of nitrated polynuclear aromatic hydrocarbons in particulate extracts by capillary column gas chromatography with nitrogen selective detection. *Anal. Chem., 55*, 1946–1954

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

Rosenkranz, H.S. & Mermelstein, R. (1983) Mutagenicity and genotoxicity of nitroarenes: all nitro-containing chemicals were not created equal. *Mutat. Res.*, *114*, 217–267

Rosenkranz, H.S. & Mermelstein, R. (1985) The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons. *J. environ. Sci. Health, C3*, 221–272

Rosenkranz, H.S., McCoy, E.C., Sanders, D.R., Butler, M., Kiriazides, D.K. & Mermelstein, R. (1980) Nitropyrenes: isolation, identification, and reduction of mutagenic impurities in carbon black and toners. *Science*, *209*, 1039–1043

Sanders, D.R. (1981) Nitropyrenes: the isolation of trace mutagenic impurities from the toluene extract of an aftertreated carbon black. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons, 5th International Symposium, Chemical Analysis and Biological Fate*, Columbus, OH, Battelle, pp. 145–158

Tokiwa, H. & Ohnishi, Y. (1986) Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *CRC crit. Rev. Toxicol.*, *17*, 23–60

Tokiwa, H., Nakagawa, R., Horikawa, K. & Ohkubo, A. (1987) The nature of the mutagenicity and carcinogenicity of nitrated, aromatic compounds in the environment. *Environ. Health Perspect.*, *73*, 191–199

Wislocki, P.G., Bagan, E.S., Lu, A.Y.H., Dooley, K.L., Fu, P.P., Han-Hsu, H., Beland, F.A. & Kadlubar, F.F. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[*a*]anthracene, chrysene and benzo[*a*]pyrene in the newborn mouse assay. *Carcinogenesis*, *7*, 1317–1322