6-NITROBENZO[*a*]PYRENE

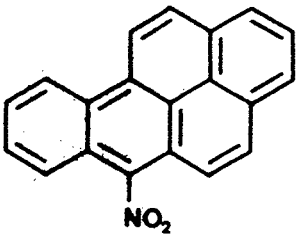
This substance was considered by a previous Working Group, in June 1983 (IARC, 1984). Since that time, new data have become available and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 63041-90-7
Chem. Abstr. Name: Benzo[*a*]pyrene, 6-nitro-
IUPAC Systematic Name: 6-Nitrobenzo[*a*]pyrene

1.2 Structural and molecular formulae and molecular weight



$C_{20}H_{11}NO_2$ Mol. wt: 297.3

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Orange-yellow needles, recrystallized from benzene (Boit, 1965); yellow crystalline solid (Chemsyn Science Laboratories, 1988); orange crystals (Buckingham, 1985)

(*b*) *Melting-point*: 250.5–251°C (Boit, 1965); 255–256°C (Buckingham, 1985)

(*c*) *Spectroscopy data*: Ultra-violet, nuclear magnetic resonance and mass spectral data have been reported (Dewar *et al.*, 1956; Fu *et al.*, 1982a; Chou *et al.*, 1984; Johansen *et al.*, 1984; Schuetzle & Jensen, 1985).

(*d*) *Solubility*: Limited solubility in toluene and benzene (Chemsyn Science Laboratories, 1988)

(*e*) *Reactivity*: Reacts with chromic acid in acetic acid to form 7-oxo-7*H*-benz[*de*]-anthracene dicarboxylic acid-3,4-anhydride (Boit, 1965)

1.4 Technical products and impurities

6-Nitrobenzo[*a*]pyrene is available at a certified purity of 99.75% (Belliardo *et al.*, 1988). It is also available at ≥99% purity and at ≥98% radiochemical purity as the ^{3}H-labelled compound (Chemsyn Science Laboratories, 1988).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

6-Nitrobenzo[*a*]pyrene was first synthesized by Windaus and Rennhak in 1937 by treating benzo[*a*]pyrene with aqueous nitric acid in either acetic acid or benzene and acetic acid (Boit, 1965). It has been reported to be formed under simulated environmental atmospheric conditions by reacting benzo[*a*]pyrene with nitrogen oxide and traces of nitric acid (Pitts *et al.*, 1978). No evidence was found that 6-nitrobenzo[*a*]pyrene has been produced in commercial quantities.

(*b*) *Use*

No evidence was found that 6-nitrobenzo[*a*]pyrene has been used for other than laboratory applications.

2.2 Occurrence

(*a*) *Engine exhaust*

Pitts *et al.* (1982) and Gibson (1982, 1983) have reported the occurrence of 6-nitrobenzo[*a*]pyrene in diesel emissions.

Illustrative values for levels of 6-nitrobenzo[*a*]pyrene found in engine emissions are given in Table 1. Pitts *et al.* (1982) reported finding 50 mg/kg in an extract and 5 mg/kg in particulate matter from the exhaust of a six-cylinder diesel-powered car. The rates of emission of 6-nitrobenz[*a*]pyrene in automobile exhaust have been reported to be 1.46 ± 0.74 μg/mile [0.9 ± 0.5 μg/km] for a gasoline-powered car with a precatalyst engine running on leaded fuel, 0.45 ± 0.22 μg/mile [0.28 ± 0.14 μg/km] for an engine with the catalyst removed running on unleaded fuel and 0.012 μg/mile [0.008 μg/km] for a gasoline-powered engine with a catalytic converter. A production model diesel engine emitted <0.15 μg/mile [<0.09 μg/km] and a diesel trap model, 0.01 μg/mile [0.006 μg/km] (Gibson, 1982).

Table 1. 6-Nitrobenzo[a]pyrene levels in exhaust particles[a]

Sample	6-Nitrobenzo[a]pyrene concentration (mg/kg particulate matter)
Gasoline-powered cars	
1974 engine (precatalyst), leaded fuel	32.8 ± 16.2
1980 engine (catalyst removed), unleaded fuel	17.3 ± 8.5
1981 engine with catalyst	0.21 ± 0.1
Diesel-powered cars	
trap model	0.1
1980 engine	<0.4

[a]From Gibson (1982)

(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).

6-Nitrobenzo[a]pyrene has been detected in airborne particles in Prague, Czechoslovakia (Jäger, 1978). Ambient particles collected in the area of Detroit, MI, USA, during the spring and summer of 1981 contained levels of 0.9–2.5 mg/kg, corresponding to airborne concentrations of 0.04–0.28 ng/m^3 (Gibson, 1982). Nielsen *et al.* (1984) detected 6-nitrobenzo[a]pyrene in airborne particles in rural Denmark during the late winter and early spring of 1982, but the levels were one to two orders of magnitude lower than those of most common polycyclic aromatic hydrocarbons such as benzo[a]pyrene. The authors suggested that formation may have occurred during sampling.

Other reported sources of emissions of 6-nitrobenzo[a]pyrene include stack gases from aluminium smelters (Oehme *et al.*, 1982). A fireplace in which red oak was burned yielded diluted emissions of 0.11 and 0.12 mg/kg particles (Gibson, 1982).

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*) *Skin application*

Mouse: In a study of initiating activity, two groups of 20 female CD-1 Charles River mice, aged 50–55 days, received ten applications of 5 μg 6-nitrobenzo[*a*]pyrene or benzo[*a*]pyrene (purity, >99%) in 0.1 ml acetone onto shaved back skin every other day for 20 days (total dose, 0.05 mg; El-Bayoumy *et al.*, 1982). A group of 20 female mice receiving acetone alone served as controls. Starting ten days after initiation had been completed, all animals received applications of 2.5 μg 12-*O*-tetradecanoylphorbol 13-acetate in 0.1 ml acetone three times per week for 25 weeks. At the end of this time, 5/20 animals treated with 6-nitrobenzo[*a*]pyrene, 18/20 benzo[*a*]pyrene-treated animals and 1/20 control animals had developed skin tumours (mainly papillomas). The incidence of papillomas in 6-nitrobenzo[*a*]pyrene-treated animals was not statistically significant from that in untreated controls [$p = 0.09$]. [The Working Group noted the small number of animals tested.]

(*b*) *Intraperitoneal administration*

Mouse: Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 6-nitrobenzo[*a*]pyrene (total dose, 560 nmol [0.17 mg]; purity, >99%) in 10, 20 and 40 μl dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth; a total dose of 560 nmol [0.14 mg] 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 males and 44 females in the treated group, 37 males and 27 females in the positive control group, 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 tumours occurred in 8/29 6-nitrobenzo[*a*]-pyrene-treated males (five adenomas, three carcinomas; $p < 0.05$), 0/44 6-nitrobenzo[*a*]-pyrene-treated females, 18/37 benzo[*a*]pyrene-treated males, 0/27 benzo[*a*]pyrene-treated females, 2/28 and 5/45 DMSO-treated males and 0/31 and 0/34 DMSO-treated females. Lung adenomas occurred in significantly more benzo[*a*]pyrene-treated males (13/37) and females (13/27) than in controls ($p < 0.005$), but no significant increase occurred among 6-nitrobenzo[*a*]pyrene-treated males (4/29) or females (1/44). The incidences of lung tumours in the DMSO groups were 1/28 and 4/45 in males and 0/31 and 2/34 in females. [The Working Group noted the short duration of the experiment.]

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

3.2 Other relevant data

(*a*) *Experimental systems*

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

Among female F344 rats administered 1 nmol [297 ng] [^{14}C]6-nitrobenzo[*a*]pyrene by intratracheal instillation, the majority of the material (86%) was cleared from the lungs with a half-life of 0.2 h, while the remaining 14% had a half-life of 36 h (Bond *et al.*, 1985).

In-vitro incubation of 6-nitrobenzo[*a*]pyrene with rat liver microsomes, 9000 *g* supernatant of rat liver or lung, rat lung microsomes or 9000 *g* supernatant of mouse liver has been reported to give rise to benzo[*a*]pyrene, 6-nitrobenzo[*a*]pyrene-7,8- and 9,10-dihydrodiols, 6-hydroxybenzo[*a*]pyrene, 1- and 3-hydroxy-6-nitrobenzo[*a*]pyrenes, 1,9-and 3,9-dihydroxy-6-nitrobenz[*a*]pyrenes, benzo[*a*]pyrene-1,6-, -3,6- and -6,12-quinones, 6-acetoxybenzo[*a*]pyrene, and mono- and diacetoxy-6-nitrobenzo[*a*]pyrenes (Fu *et al.*, 1982a,b; Chou *et al.*, 1983; Raha *et al.*, 1984, 1986a,b, 1987a,b). Similar incubation with 9000 *g* supernatant of liver from rats pretreated with 3-methylcholanthrene led to binding to exogenous DNA (Kaneko & Nagata, 1983).

Anaerobic bacterial suspensions from human faeces and intestinal contents, rat faeces and intestinal contents and pure cultures of anaerobic bacteria reduced 6-nitrobenzo[*a*]-pyrene to 6-aminobenzo[*a*]pyrene (Cerniglia *et al.*, 1984; Richardson *et al.*, 1988).

6-Nitrobenzo[*a*]pyrene was reduced to metabolites soluble in organic solvents (predominant presence of dihydrodiols) and in water by hamster embryo fibroblasts. The metabolism was accompanied by binding to RNA, DNA and nuclear protein (Selkirk *et al.*, 1981; Tong & Selkirk, 1983).

Incubation of explants of human colon and bronchus with 6-nitrobenzo[*a*]pyrene resulted in the formation of one major metabolite, 3-hydroxy-6-nitrobenzo[*a*]pyrene, which bound to the DNA of both tissues. Two adducts were identified in bronchial DNA, one of which had a retention time in high-performance liquid chromatograms similar to that of the major adduct found in lung and liver DNA of Sprague-Dawley rats treated with an intraperitoneal dose of 2 mg/kg bw 6-nitrobenzo[*a*]pyrene. By comparison, three adducts were detected in the DNA of *Salmonella typhimurium* TA98 that had been incubated with 6-nitrobenzo[*a*]pyrene in the presence of an exogenous metabolic system from rat liver. One of these adducts appeared to have a similar retention time to the major adduct detected in rat lung and liver (Garner *et al.*, 1985).

(ii) *Toxic effects*

Intraperitoneal administration of 6-nitrobenzo[*a*]pyrene to young male Sprague-Dawley rats (three times at 5 mg/kg bw) resulted in a 1.6-fold increase in aryl hydrocarbon hydroxylase activity over that in controls (Chou *et al.*, 1987).

(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).

6-Nitrobenzo[*a*]pyrene (1–5 nmol/plate) was mutagenic to *S. typhimurium* TA98 and TA100, generally only in the presence of an exogenous metabolic system from rat liver (Wang *et al.*, 1978; Tokiwa *et al.*, 1981; Fu *et al.*, 1982a,b; Pitts *et al.*, 1982; Chou *et al.*, 1984; Greibrokk *et al.*, 1984; Fu *et al.*, 1985; White *et al.*, 1985; Hass *et al.*, 1986a; Löfroth *et al.*, 1984; Anderson *et al.*, 1987).

Early studies indicated that 6-nitrobenzo[*a*]pyrene was mutagenic to the *hprt* locus of cultured Chinese hamster CHO cells in the absence of an exogenous metabolic system (1–3 μg/ml; Chou *et al.*, 1984), but subsequent reports indicated positive results in the presence (one dose only, 5 μg/ml) and negative results in the absence (up to 10 μg/ml) of metabolic activation (Hass *et al.*, 1986b).

6-Nitrobenzo[*a*]pyrene induced morphological transformation in cultured Syrian hamster embryo cells (DiPaolo *et al.*, 1983 (6.6–34 μM); Sala *et al.*, 1987 (marginally active at 2–8 μM)) and transformation (induction of growth in soft agar and invasiveness in chicken embryo skin culture) in human diploid fibroblasts when oxygen tension was reduced (Howard *et al.*, 1983 (4–67 μM)) and in mouse BALB/c 3T3 cells (Sala *et al.*, 1987 (4–32 μM)). It did not induce transformation of mouse C3H 10T1/2 cells (at up to 8 μM), nor did it act as an initiator in these cells (Sala *et al.*, 1987).

(*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

6-Nitrobenzo[*a*]pyrene has been detected in stack gases from aluminium smelters and in particulate emissions from diesel and gasoline engines. It has also been measured at low concentrations in ambient air.

4.2 Experimental data

6-Nitrobenzo[*a*]pyrene was tested for carcinogenicity in a two-stage initiation-promotion study in mouse skin and by intraperitoneal injection into newborn mice. The study by skin application was inadequate for evaluation. An increased incidence of liver-cell tumours was observed in male mice after intraperitoneal injection.

4.3 Human data

No data were available to the Working Group.

4.4 Other relevant data

Metabolism of 6-nitrobenzo[*a*]pyrene led to DNA adduct formation in bacteria, explanted human tissues and animals. 6-Nitrobenzo[*a*]pyrene caused transformation of animal and human cultured cells and was mutagenic to cultured animal cells. It induced mutations in bacteria in the presence of an exogenous metabolic system.

4.5 Evaluation[1]

There is *limited evidence* for the carcinogenicity in experimental animals of 6-nitrobenzo[*a*]pyrene.

No data were available from studies in humans on the carcinogenicity of 6-nitrobenzo[*a*]-pyrene.

Overall evaluation

6-Nitrobenzo[*a*]pyrene *is not classifiable as to its carcinogenicity to humans (Group 3).*

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

Summary table of genetic and related effects of 6-nitrobenzo[*a*]pyrene

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

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