6-NITROCHRYSENE

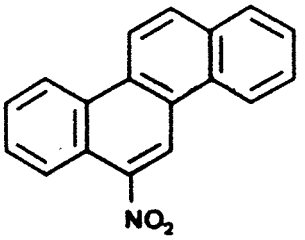
This substance was considered by a previous Working Group, in June 1983 (IARC, 1984). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 7496-02-8
Chem. Abstr. Name: Chrysene, 6-nitro
IUPAC Systematic Name: 6-Nitrochrysene

1.2 Structural and molecular formulae and molecular weight



$C_{18}H_{11}NO_2$ Mol. wt: 273.3

1.3 Chemical and physical properties of the pure substance

(*a*) *Description*: Chrome-red, thick prismatic crystals (Prager & Jacobson, 1922); orange-yellow needles (Boit, 1965); light-yellow needles (Chemsyn Science Laboratories, 1988)

(*b*) *Boiling-point*: Sublimes without decomposition (Prager & Jacobson, 1922)

(*c*) *Melting-point*: 209°C (Boit, 1965)

(*d*) *Spectroscopy data*: Mass spectral data have been reported (Schuetzle & Jensen, 1985).

(*e*) *Solubility*: Slightly soluble in cold ethanol, diethyl ether and carbon disulfide; somewhat more soluble in benzene and acetic acid; soluble in hot nitrobenzene (Prager & Jacobson, 1922)

(*f*) *Reactivity*: Forms 6-aminochrysene upon heating with tin and concentrated hydrochloric acid in acetic acid at 100°C. Reacts with bromine to form 12-bromo-6-nitrochrysene, and reacts with fuming nitric acid to form 6,12-dinitrochrysene (Boit, 1965)

1.4 Technical products and impurities

6-Nitrochrysene is offered for sale in research quantities at ≥98% purity (Chemsyn Science Laboratories, 1988) and is also available at a certified purity of 98.9% as a reference material (Belliardo *et al.*, 1988).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

6-Nitrochrysene was first synthesized in 1890 by heating chrysene with aqueous nitric acid in acetic acid (Prager & Jacobson, 1922). It can also be synthesized by briefly heating chrysene with nitric acid and concentrated sulfuric acid in acetic acid at 40°C (Boit, 1965).

(*b*) *Use*

No evidence was found that 6-nitrochrysene has been used in commercial applications. It is used as an internal standard in the chemical analysis of nitroarenes (see monograph on 1-nitropyrene).

2.2 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).

Garner *et al.* (1986) identified 6-nitrochrysene at a level of ~1 ng/m^3 in ambient air in Upper Frankonia, Federal Republic of Germany. Atmospheric transformation of chrysene to mononitrochrysene is reported to occur in the presence of 19 mg/m^3 nitrogen dioxide (Tokiwa & Ohnishi, 1986).

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, a group of 20 female CD-1 Charles River mice, aged 50–55 days, received ten applications of 0.1 mg 6-nitrochrysene (purity, >99%) in 0.1 ml acetone onto shaved back skin every other day for 20 days (total dose, 1 mg; El-Bayoumy *et al.*, 1982). Another group of 20 female mice receiving acetone alone served as controls. Starting ten days after the initiation treatment 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, 12/20 of the treated animals had developed skin tumours (mainly papillomas; 2.1 tumours/animal) compared with 1/20 controls ($p < 0.01$).

(*b*) *Intraperitoneal administration*

Mouse: A group of 22 male and 29 female newborn Swiss-Webster BLU-Ha mice received three intraperitoneal injections of 6-nitrochrysene (total dose, 38.5 μg/mouse [purity unspecified] in 5, 10 and 20 μl dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth (Busby *et al.*, 1985). Another group of 23 male and 21 female newborn mice received three injections to give a total dose of 189 μg 6-nitrochrysene. A further group of 22 males and 15 females received injections of DMSO only and served as controls. Animals were killed and necropsied at 26 weeks of age. All mice injected with 6-nitrochrysene developed multiple lung tumours, the incidence of which was significantly different from that in controls (three males and one female with lung tumours; $p < 0.0001$); 70% of treated animals had adenocarcinomas. Control mice with tumours of the lung did not develop more than two adenomas per mouse, but the average number of lung tumours was increased by 150

[1]The Working Group was aware of a study in progress in mice by single subcutaneous injection (IARC, 1988). Subsequent to the meeting, the Secretariat became aware of a study in newborn mice by intraperitoneal injection in which lung tumours were induced in animals of each sex and liver tumours were induced in males (El-Bayoumy *et al.*, 1989).

and 250 fold in the two treated groups, respectively. Female mice in both treatment groups developed ~40% more lung tumours than males, although this difference was not statistically significant. A few lymphomas and nodular hyperplasia of the liver were observed in treated but not in control animals.

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 6-nitrochrysene (total dose, 2800 nmol [0.77 mg]; purity, >99%) in 10, 20 and 40 μl DMSO on days 1, 8 and 15 after birth; a total dose of 700 nmol [0.2 mg] 6-nitrochrysene; 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 and of the group administered 700 nmol 6-nitrochrysene was begun ten weeks after that of the other groups. At 25–27 days, when the mice were weaned, nine males and 11 females in the group that received 2800 nmol 6-nitrochrysene, 33 males and 40 females in the group that received 700 nmol 6-nitrochrysene, 37 males and 27 females in the positive control group, and 28 and 31 males and 45 and 34 females in the vehicle control groups were still alive. All remaining mice were killed after one year. Liver-cell tumours occurred in 3/9 males given 2800 nmol (carcinomas; $p < 0.05$), in 3/11 females given 2800 nmol (two adenomas, one carcinoma; $p < 0.05$); in 25/33 males given 700 nmol (one adenoma, 24 carcinomas; $p < 0.05$); in 9/40 females given 700 nmol (five adenomas, four carcinomas; $p < 0.005$); in 2/28 and 5/45 DMSO-treated males and in 0/31 and 0/34 DMSO-treated females. Treatment with 700 nmol 6-nitrochrysene increased the multiplicity of hepatic nodules per tumour-bearing mouse. Lung tumours occurred in 7/9 males given 2800 nmol, 9/11 females given 2800 nmol, 28/33 males given 700 nmol (11 adenomas, 17 carcinomas) and 36/40 females given 700 nmol (19 adenomas, 17 carcinomas); all of these incidences were statistically significantly different ($p < 0.005$) from those in DMSO controls (1/28 and 4/45 in males and 0/31 and 2/34 in females). The numbers of mice with malignant lymphomas were substantially increased in both groups administered 6-nitrochrysene ($p < 0.005$ for the 700 nmol group). In the group given benzo[*a*]pyrene, 18/37 males had hepatic tumours and 13/37 had lung adenomas, and 13/27 females had lung adenomas; no significant increase in the incidence of malignant lymphomas was observed.

3.2 Other relevant data

(*a*) *Experimental systems*

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

Incubation of 6-nitrochrysene with an exogenous metabolic system from rat liver has been reported to result in the formation of *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene and 6-aminochrysene (El-Bayoumy & Hecht, 1984). Anaerobic bacteria from human faeces metabolized 6-nitrochrysene to 6-nitrosochrysene, 6-aminochrysene, *N*-formyl-6-aminochrysene and 6-acetyl aminochrysene. Mixed cultures of rat and mouse intestinal bacteria and pure cultures of anaerobic bacteria reduced 6-nitrochrysene to 6-aminochrysene (Manning *et al.*, 1988).

Incubation of primary cultures of rat liver hepatocytes with 6-nitrochrysene resulted in the formation of two DNA adducts, *N*-(deoxyguanosin-8-yl)-6-aminochrysene and *N*-(deoxyinosin-8-yl)-6-aminochrysene. The latter adduct was suggested to arise from the deamination of *N*-(deoxyadenosin-8-yl)-6-aminochrysene. The same two adducts plus 5-(deoxyguanosin-N^2-yl)-6-aminochrysene were formed by reacting *N*-hydroxy-6-aminochrysene with DNA (Delclos *et al.*, 1987a).

After preweanling male CD mice were given one or three intraperitoneal doses of 6-nitrochrysene, analysis of lung and liver DNA indicated a single major adduct that corresponded to the adduct detected after the microsomal incubation of 6-aminochrysene *trans*-1,2-dihydrodiol with calf thymus DNA (Delclos *et al.*, 1988). The same adduct was found at lower levels in mice treated with 6-aminochrysene (Delclos *et al.*, 1987b).

As reported in an abstract, 6-nitrochrysene was incubated with liver microsomes from preweanling BLU:Ha mice, from 3-methylcholanthrene-pretreated Sprague-Dawley rats and from Aroclor-pretreated Fischer rats, and with human lung explants and lung and liver microsomes. 6-Nitrochrysene *trans*-1,2-dihydrodiol and 6-nitrochrysene *trans*-9,10-dihydrodiol were found as metabolites. The metabolic profile detected with the human lung explants and in preweanling mice was similar to that observed in the microsomal incubations. A DNA adduct was detected in the human lung explants that appeared to be identical to that previously found in mouse liver DNA (Delclos *et al.*, 1987c).

(ii) *Toxic effects*

Intraperitoneal administration of a total dose of 2800 nmol 6-nitrochrysene to newborn CD mice increased mortality at weaning by two fold (Wislocki *et al.*, 1986).

Two intraperitoneal administrations of 5 mg/kg bw 6-nitrochrysene to young male Sprague-Dawley rats resulted in an eight-fold increase in aryl hydrocarbon hydroxylase activity over that in controls; two injections of 2.5 mg/kg bw resulted in a 4.5-fold increase. Three-fold increases in the activities of 7-ethoxycoumarin-*O*-deethylase and 1-nitropyrene reductase were seen at the lower dose (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-Nitrochrysene (0.5 μg/disc) preferentially inhibited the growth of DNA repair-deficient *Bacillus subtilis* (Tokiwa *et al.*, 1987) and was mutagenic to *Salmonella typhimurium* TA98 and TA100 (2 μg/plate; Pederson & Siak, 1981; Tokiwa *et al.*, 1981a,b; Sugimura & Takayama, 1983; El Bayoumy & Hecht, 1984; Greibrokk *et al.*, 1984).

6-Nitrochrysene induced morphological transformation in cultured Syrian hamster embryo cells (DiPaolo *et al.*, 1983 (3.7–73 μM); Sala *et al.*, 1987 (3.6–10.8 μg/ml)). It did not induce transformation in murine BALB/c 3T3 (at up to 40 μM) or C3H 10T1/2 cells (at up to 55 μM; 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-Nitrochrysene was found in ambient air at a low concentration in one study.

4.2 Experimental data

6-Nitrochrysene was tested for initiating activity on mouse skin and was found to be active. It was also tested for carcinogenicity in two experiments by intraperitoneal injection into newborn mice, producing increased incidences of lung and liver-cell tumours and of malignant lymphomas.

4.3 Human data

No data were available to the Working Group.

4.4 Other relevant data

Intraperitoneal injection of 6-nitrochrysene caused a substantial increase in aryl hydrocarbon hydroxylase activity in rat liver. Metabolism of 6-nitrochrysene led to DNA adduct formation in cultured mammalian cells and in animals. 6-Nitrochrysene caused transformation in cultured animal cells. It was mutagenic to and induced DNA damage in bacteria.

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity in experimental animals of 6-nitrochrysene.

No data were available from studies in humans on the carcinogenicity of 6-nitrochrysene.

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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-nitrochrysene

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]										

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

Overall evaluation

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

5. References

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