1-NITROPYRENE

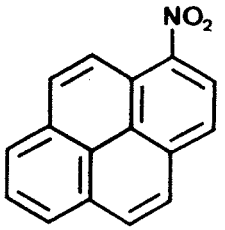
This substance was considered by a previous Working Group, in June 1983 (IARC, 1984). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 5522-43-0
Chem. Abstr. Name: Pyrene, 1-nitro-
IUPAC Systematic Name: 1-Nitropyrene
Synonym: 3-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*: Yellow needles or prisms from ethanol (Prager & Jacobson, 1922)

(*b*) *Melting-point*: 155°C (Luckenbach, 1980)

(*c*) *Spectroscopy data*: Ultra-violet (Bavin & Dewar, 1955; Paputa-Peck *et al.*, 1983), nuclear magnetic resonance (Kaplan, 1981; Paputa-Peck *et al.*, 1983) and mass (Schuetzle & Jensen, 1985) spectral data have been reported.

(*d*) *Solubility*: Very soluble in diethyl ether (Prager & Jacobson, 1922); soluble in ethanol and benzene at 15°C (Luckenbach, 1980); soluble in toluene and tetrahydrofluorenone (Chemsyn Science Laboratories, 1988)

(*e*) *Reactivity*: Reacts with ethanolic potassium hydroxide to form 1,1′-azoxypyrene; also reacts with zinc powder in ethanol in the presence of catalytic amounts of ammonium chloride or ammonia to form 1,1′-azoxypyrene or, without air, 1-aminopyrene and 1-hydroxylaminopyrene (Boit, 1965)

(*f*) *Stability*: Photodecomposition to 2-propanol is readily induced by ultra-violet/-visible light (Stärk *et al.*, 1985).

1.4 Technical products and impurities

1-Nitropyrene is available for research purposes at 97% (Aldrich Chemical Co., 1988) or ≥99.5% purity with ≤0.1% total dinitropyrenes and pyrene (Chemsyn Science Laboratories, 1988). It is available at a purity of 99.68% as a reference material (Belliardo *et al.*, 1988).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

1-Nitropyrene was first synthesized by Graebe in 1871 by heating pyrene with equal parts of nitric acid and water. It can also be obtained (in a mixture with dinitropyrenes) by the addition of potassium nitrite to a solution of pyrene in diethyl ether, followed by the slow addition of dilute sulfuric acid (Prager & Jacobson, 1922). This compound has also been synthesized by heating pyrene with nitric acid in acetic acid at 50°C (Boit, 1965). 1-Nitropyrene was the only mononitropyrene isomer produced when pyrene was reacted with nitrogen pentoxide in carbon tetrachloride, and only traces of other isomers were found (Pitts *et al.*, 1985).

1-Nitropyrene is formed as a result of the photochemical oxidation of 1-aminopyrene induced by ultra-violet A irradiation (Okinaka *et al.*, 1986).

Since 1972, one Japanese company has produced this compound by the reaction of pyrene with nitric acid. 1-Nitropyrene is reported in the 1985 *Toxic Substances Control Act Chemical Substance Inventory* (US Environmental Protection Agency, 1986).

(*b*) *Use*

1-Nitropyrene has been reported to be a chemical photosensitizer, increasing the spectral sensitivity of bis-azide compounds in the long-wavelength region (Tsunoda *et al.*, 1973). It

has been reported that one Japanese company uses 1-nitropyrene as an intermediate in the production of 1-azidopyrene, which is used in photosensitive printing.

2.2 Occurrence

(*a*) *Engine exhaust*

1-Nitropyrene is one of the major nitroarenes in primary particulate emissions of diesel engines (Pitts, 1987). Substantially decreased amounts of 1-nitropyrene were reported in exhausts emitted from a single cylinder when nitrogen-free air was used in the diesel engine (Herr *et al.*, 1982). Illustrative data on 1-nitropyrene levels in the particles of exhaust emissions and in extracts of these particles are summarized in Table 1.

1-Nitropyrene has also been identified in used oil from a light-duty diesel engine at levels of 0.2 mg/kg after 8000 km and 0.5 mg/kg after 9000 km (Jensen *et al.*, 1986), and Manabe *et al.* (1984) found 0.4 mg/kg in used (4600 km) diesel engine oil and 0.2 mg/kg in used (3200 km) gasoline engine oil. Jensen *et al.* (1986) reported that oil was the source of a significant amount (16–80% depending on engine load) of extractable organic materials in diesel particulate emissions. Since 1-nitropyrene was not detected in new oil (on the basis of a detection limit of 0.1 mg/kg), they postulated that the nitropyrene found in used oil represents formation, scavenging during combustion or accumulation of the compound in oil during use. They concluded that the emission rate of 1-nitropyrene increases as oil ages with use. Emission rates of 1-nitropyrene in particles in vehicle engine exhausts are given in Table 2.

(*b*) *Other occurrence*

1-Nitropyrene is one of the most abundant mononitroarenes in the ambient atmosphere. Quantitative data on 1-nitropyrene levels in samples of airborne particulate matter are summarized in Table 3.

Nitroarenes occur in the emissions of numerous stationary combustion sources. 1-Nitropyrene was identified in Norway at more than 100 times the typical ambient air concentration in a potroom where Söderberg electrodes were used for aluminium reduction (Thrane & Stray, 1986). In addition, 1-nitropyrene was detected in stack gases from aluminium smelters and wood stoves in Denmark (Nielsen *et al.*, 1984) and in simulated stack gas. It was concluded that 1-nitropyrene is formed by reaction of pyrene in the presence of nitrogen and sulfur oxides during the sampling process (Brorström-Lundén & Lindskog, 1985).

1-Nitropyrene has been identified in coal fly-ash (Mumford & Lewtas, 1982), in fly-ash extracts of the combustion products of western low-sulfur coal collected in the stack of a commercial power plant (Harris *et al.*, 1984), and in both gas-phase and particulate condensates of flue gases from several coal-fired energy conversion plants (Olsen *et al.*, 1984). Since polycyclic aromatic hydrocarbons (PAH) were found mostly in the gaseous phase, it was concluded by the Working Group that the 1-nitropyrene originated in the oxidizer/combustion unit. 1-Nitropyrene was found in particles emitted from a wood

Table 1. 1-Nitropyrene levels in exhaust particles and their extracts

Sample	1-Nitropyrene concentration		Reference
	mg/kg extract	mg/kg particulate matter	
Diesel			
Car	–	8.6	Morita *et al.* (1982)
1978 production model	–	3.9	Gibson (1983)
1980 production model	–	8.0 ± 2.4	Gibson (1982)
1982 production model	–	7.6–24.5	Gibson (1983)
Mixed cars	–	9.1	Gibson (1983)
4-Stroke, 6-cyclinder engines, typical of long-distance trucks	<2–39	–	Rappaport *et al.* (1982)
6-Cylinder engine	870	93	Pitts *et al.* (1982)
Passenger vehicle	55 ± 11 150 ± 30	–	Salmeen *et al.* (1982)
1979 Passenger vehicle	2030 ± 220	–	Salmeen *et al.* (1982)
Light-duty engine	2280 ± 230	–	Schuetzle *et al.* (1982)
Passenger car	75 ± 10	–	Salmeen *et al.* (1984)
Bus	70.5	30[a]	Nakagawa *et al.* (1983)
Passenger car	107.2–589.3	–	Nishioka *et al.* (1982)
Diesel-trap car	–	14.2	Gibson (1982)
Heavy-duty diesel (commercial mining engine)			Draper (1986)
100% load, 1200 rpm	–	<0.12	
75% load, 1800 rpm	–	5.0	
Diesel vehicles (on road/mountain tunnel, Pennsylvania)	–	2.1	Gorse *et al.* (1983)
Gasoline			
Catalyst engine	–	0.63 ± 0.52	Gibson (1982)
No catalyst/unleaded		4.3 ± 3.2	
No catalyst/leaded	–	3.9 ± 1.3	Gibson (1982)
Passenger car	2.5	–	Nishioka *et al.* (1982)
Spark ignition vehicles 75% catalyst equipped (on road/mountain tunnel, Pennsylvania)	–	5	Gorse *et al.* (1983)

[a]Calculated by the Working Group for comparative purposes from data in the reference

Table 2. Emission rates of 1-nitropyrene in exhaust particles of diesel and gasoline vehicles

Sample[a]	1-Nitropyrene particulate phase emission rate		Reference
	μg/km[b]	μg/mile	
Diesel			
Production model car	(2.0 ± 0.8)	3.2 ± 1.2	Gibson (1982)
Diesel-trap car	(0.8)	1.2	Gibson (1982)
LDD/urban simulation (FTP)	4.6	–	Gorse *et al.* (1983)
LDD/highway simulation (HWFET)	4.2	–	Gorse *et al.* (1983)
HDD/direct-injection engines on road (Pennsylvania)	0.49 ± 0.06	–	Gorse *et al.* (1983)
LDD 22% fuel aromaticity	(2.6)	4.1	Schuetzle & Frazier (1986)
55% fuel aromaticity	(2.3)	3.7	
Gasoline			
No catalyst car	(0.13 ± 0.08)	0.20 ± 0.13[c]	Lang *et al.* (1981)
No catalyst/unleaded	(0.06 ± 0.06)	0.10 ± 0.09	Gibson (1982)
No catalyst/leaded	(0.11 ± 0.03)	0.17 ± 0.05	Gibson (1982)
Catalyst	(0.15 ± 0.26)	0.24 ± 0.41[c]	Lang *et al.* (1981)
Catalyst	(0.03)	0.05	Gibson (1982)
Spark-ignition cars	0.03	–	Gorse *et al.* (1983)

[a]LDD, light-duty diesel engine; FTP, Federal Test Procedure; HWFET, Highway Fuel Economy Test; HDD, heavy-duty diesel engine

[b]Figures in parentheses are conversions from reported figures to μg/km.

[c]Nitropyrene unspecified, presumed to be 1-nitropyrene

fireplace (0.11 mg/kg; Gibson, 1982) and from a coal-fired boiler (0.18 mg/kg; Gibson, 1983).

1-Nitropyrene was quantified in crude extracts of particles from gas burners (20.6 mg/kg) and from liquefied petroleum gas (LPG) burners (1.88 mg/kg), which are used widely for home heating and cooking in Japan (Tokiwa *et al.*, 1985). 1-Nitropyrene was detected in Japanese grilled chicken (*yakitori*); the level varied with grilling time at 3.8, 19 and 43 μg/kg for 3, 5 and 7 min, respectively (Kinouchi *et al.*, 1986a).

Toners for use in photocopy machines have been produced in quantity since the late 1950s and have seen widespread use. 'Long-flow' furnace black toner was first used in photocopy toners in 1967; its manufacture involved an oxidation process whereby 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).

Table 3. 1-Nitropyrene levels in atmospheric airborne particles and air samples

Sample	1-Nitropyrene concentration		Reference
	mg/kg particulate matter	ng/m³ air sample	
Detroit, MI area, summer	0.2–0.6	0.016–0.030	Gibson (1982)
Japan, industrial	–	0.021	Morita *et al.* (1982)
Santiago, Chile, winter 1981	0.06–0.15	0.028–0.110	Tokiwa *et al.* (1983)
Japan, industrial			Tokiwa *et al.* (1983)
spring	–	0.072	
summer	–	0.022	
autumn	–	0.051	
winter	–	0.045	
Denmark, rural winter, 1982	–	<0.001–0.04	Nielsen (1983)
Tunnel air (Allegheny mountain tunnel, PA)	–	0.04–0.12	Gorse *et al.* (1983)
Oslo, Norway, urban	–	0.01–0.22	Thrane & Stray (1986)
Tokyo, Japan, urban	0.19–1.6	0.015–0.134	Tanabe *et al.* (1986)
Michigan, urban, summer	0.04–0.11	0.002–0.012	Siak *et al.* (1985)
Riverside, CA, summer, 1984	–	0.008–0.03	Pitts (1987)
Aurskog, Norway, winter 1984	0.15	–	Ramdahl *et al.* (1986)
Claremont, CA, summer, 1985	0.36	–	Ramdahl *et al.* (1986)
St Louis, MO	0.16	–	Ramdahl *et al.* (1986)
Washington DC	0.20	–	Ramdahl *et al.* (1986)
Bermuda, remote			Gibson (1986)
summer, 1982	0.52 ± 0.29	0.010	
winter, 1983	0.72 ± 0.43	0.010	
Delaware, rural, summer, 1982	0.54 ± 0.24	0.013	Gibson (1986)
Warren, MI, suburban			Gibson (1986)
winter, 1982	0.36 ± 0.15	0.015	
summer, 1984	0.35 ± 0.12	0.022	
Detroit, MI, urban, summer, 1981	0.22 ± 0.20	0.030	Gibson (1986)
River Rouge, MI, industrial, summer, 1982	0.59 ± 0.56	0.057	Gibson (1986)
Dearborn, MI, industrial, summer, 1980	0.15 ± 0.13	0.029	Gibson (1986)
Torrance, CA, winter			Arey *et al.* (1987)
day-time	–	0.04	
night-time	–	0.03	

1-Nitropyrene was found at a level of 2.9 mg/kg in an extract of a pre-1979 sample of furnace black that had been aftertreated by an oxidation-nitration process (Sanders, 1981). One lot of this grade made in 1980 was found to contain 0.067 mg/kg mononitropyrene (Giammarise *et al.*, 1982). In a more recent study, an undetermined level of 1-nitropyrene was detected in an extract of a formerly available commercial furnace black produced before 1980 (Ramdahl & Urdal, 1982).

1-Nitropyrene has been detected in the waste-water from gasoline service stations (Manabe *et al.*, 1984) and in river sediment, at 25.2 μg/kg sediment (Sato *et al.*, 1985).

2.3 Analysis

This section applies to nitroarenes in general.

(*a*) *Sampling and extraction*

The sampling and extraction of nitroarenes from exhausts are described in the monograph on diesel and gasoline engine exhausts; the topic has also been reviewed by Chan and Gibson (1985).

(*b*) *Clean-up and separation of samples containing nitroarenes*

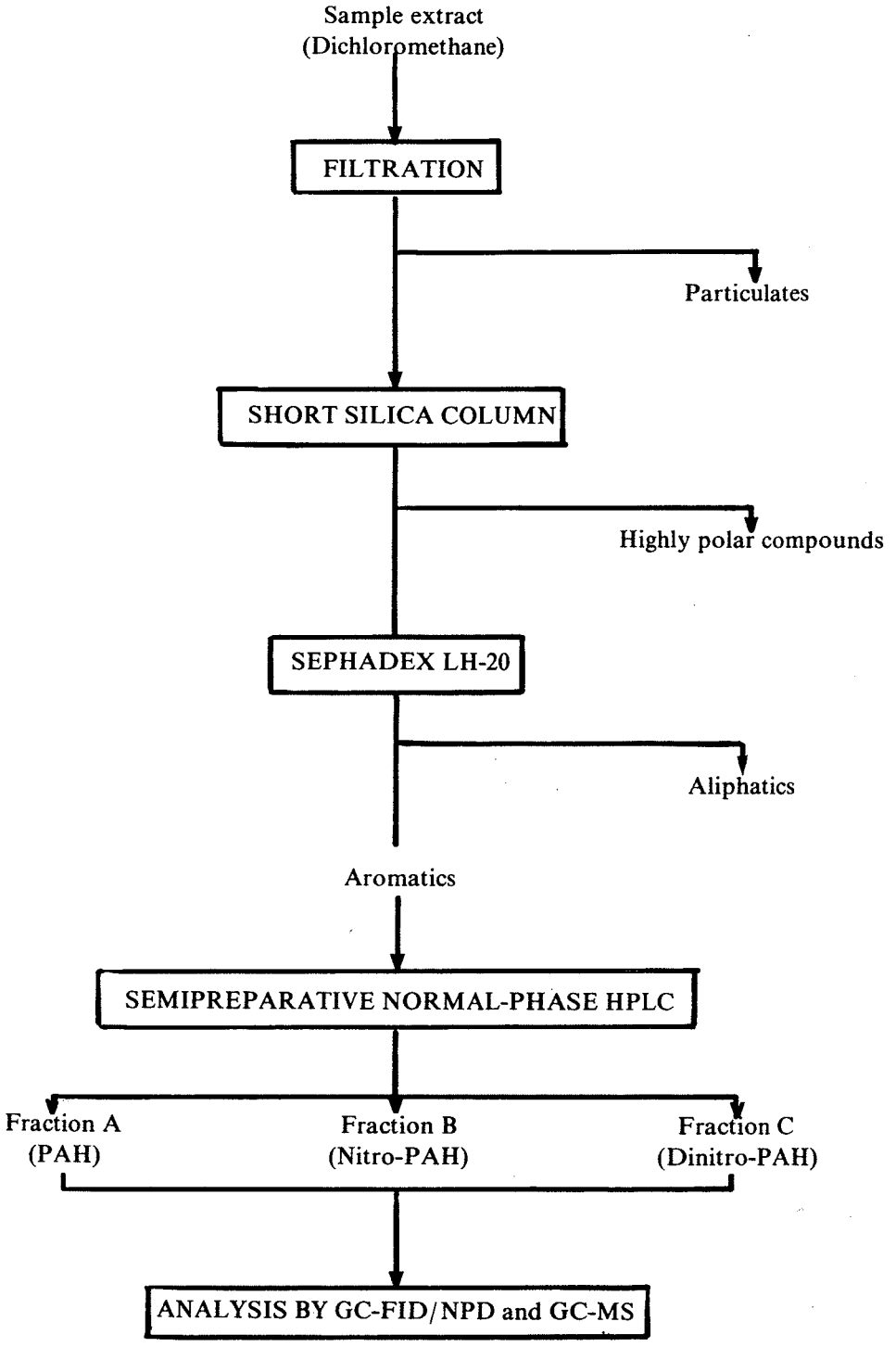
In most enrichment procedures, nitroarenes appear in the so-called 'PAH fraction'. This fraction can be separated further by column chromatography on silica gel (Grimmer *et al.*, 1987) or, more efficiently, by high-performance liquid chromatography (HPLC; Nielsen, 1983) using, e.g., normal-phase HPLC with silica gel columns (Nucleosil-Si-50-5) at room temperature with *n*-hexane:benzene (3:1) as eluent. Relative retention times (anthracene = 1.00) of 2.1–3.7 were found for mononitroarenes, which allows good separation from PAH, which have retention times of 0.78–1.26. Dinitroarenes have significantly longer retention times; other polar compounds such as cyano derivatives and aldehydes may interfere in the analysis.

A separation method for nitroarenes, consisting of silica gel filtration, chromatography on Sephadex LH 20 and subsequent semipreparative normal-phase HPLC, allows the fractionation of PAH, nitroarenes and dinitroarenes (D'Agostino *et al.*, 1983; Fig. 1). Using sodium borohydride and cupric chloride, nitroarenes are converted into the corresponding amines, which are readily separable from PAH by chromatography on silica gel (Gibson *et al.*, 1981). Another advantage of this method is that aminoarenes exhibit intense fluorescence spectra which facilitate their detection. This method has also been used to derivatize propionates from the corresponding aminoarenes with pentafluoropropionic anhydride (Fig. 2); propionates give high signal responses when an electron-capture detector is used with gas chromatography (Campbell & Lee, 1984).

(*c*) *Chemical analysis*

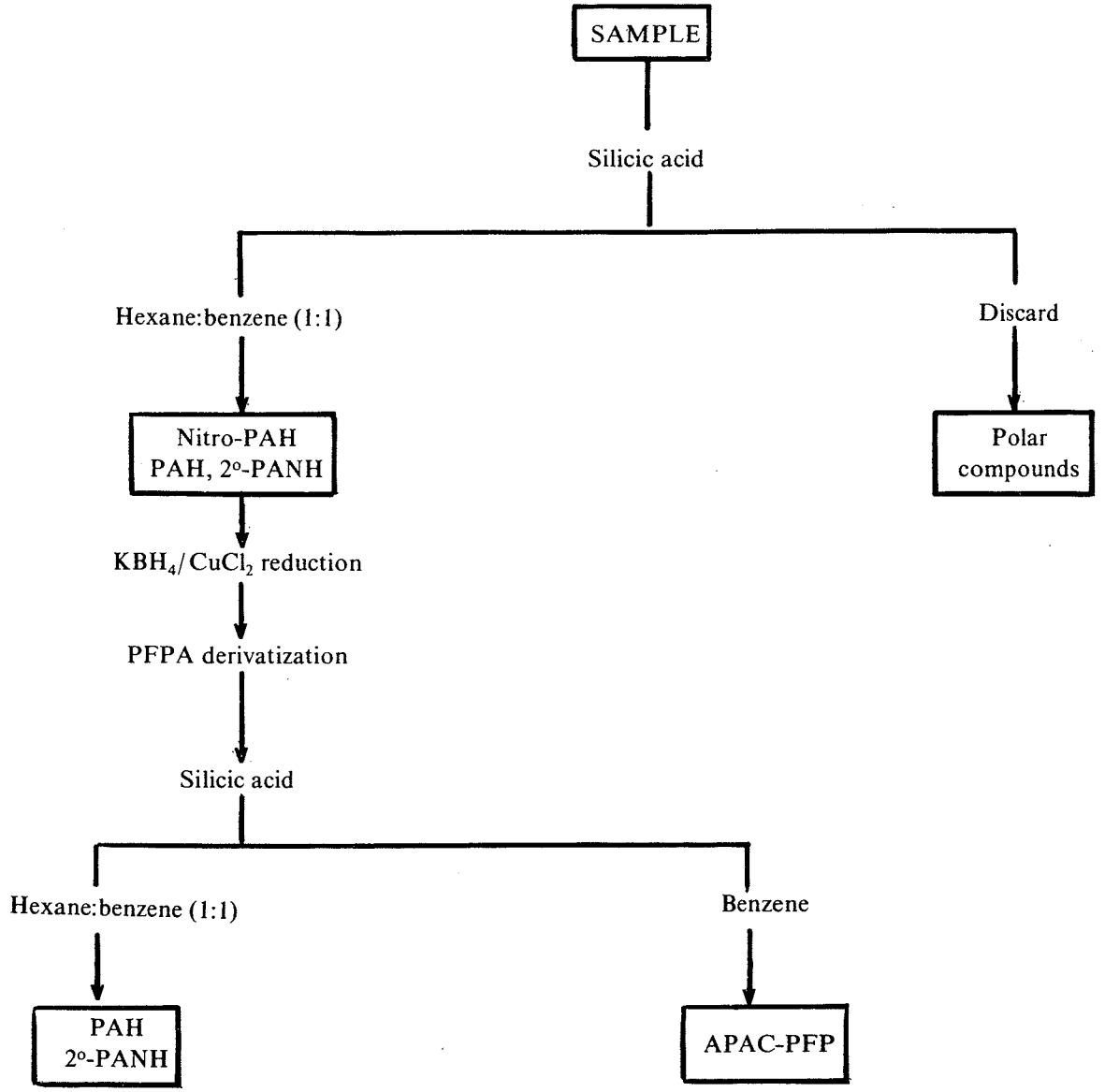
Nitroarenes have been analysed by HPLC, gas chromatography and mass spectrometry; some thin-layer chromatography methods have also been described (e.g., Pitts *et al.*, 1978).

Fig. 1. Scheme for the isolation of polycyclic aromatic hydrocarbons (PAH) and nitroarenes in environmental samples[a]



[a]From D'Agostino *et al.* (1983); HPLC, high-performance liquid chromatography; GC-FID/NPD, gas chromatography-flame ionization detection/nitrogen phosphorous detection; GC-MS, gas chromatography-mass spectrometry

Fig. 2. Scheme for the separation of nitroarenes[a]



[a]From Campbell & Lee (1984); nitro-PAH, nitroarenes; PAH, polycyclic aromatic hydrocarbons; 2°-PANH, secondary azaarenes (e.g., carbazoles); PFPA, pentafluoropropionic anhydride; APAC-PFP, pentafluoropropylamide derivatives of aminoarenes

(i) *High-performance liquid chromatography*

Conditions for analytical, preparative and semipreparative liquid chromatography have been reviewed (Poole, 1985). Supports in microbore (packed microtubular), packed capillary and open tubular microcolumns using normal and reverse-phase HPLC have been used. Due to the poor sensitivity of ultra-violet detection, more sensitive and selective fluorescent detectors are favoured. Responses can be greatly increased by means of reductive electrochemical detection, which allows quantification over a linear range of 10^3 with a sensitivity of 10–100 pg per compound (Rappaport *et al.*, 1982; Jin & Rappaport, 1983; MacCrehan & May, 1984). Conversion of nitroarenes by sodium borohydride/cupric chloride reduction to aminoarenes has also been used to increase detection sensitivity

(Gibson *et al.*, 1981). Chiral stationary phases have been introduced into HPLC for the separation of geometric isomeric nitroarenes and their derivatives (Chou, 1986).

(ii) *Gas chromatography*

The various parameters involved in the gas chromatography of nitroarenes (support, stationary phase, working conditions) and in the relative retention times of many individual nitroarenes have been reviewed (White, 1985), together with the advantages of different detectors (Tomkins, 1985). Both the common carbon-dependent flame-ionization detector and nitrogen-phosphorous detectors have been used widely for the detection of nitroarenes, sometimes in combination (Ramdahl *et al.*, 1982; Nielsen, 1983; Nielsen *et al.*, 1983). Electron-capture detectors have been used preferentially when nitroarenes have been converted previously to aminoarenes and derivatized with either heptafluorobutyric anhydride (Morita *et al.*, 1982) or pentafluoropropionic anhydride to the corresponding amides. Increased responses can be obtained when a thermionic ionization detector is used (Patterson *et al.*, 1982). Further progress has been made by introducing the thermal energy analyser, which is highly selective for nitroarenes. Optimal responses were obtained at $\geqslant$800°C pyrolyser temperature, and detection limits of 30–80 pg were reported for mononitroarenes and of 25 pg for trinitro compounds (Yu, 1983).

(iii) *Mass spectrometry*

The use of mass spectrometry in the detection of nitroarenes has been reviewed, and the relative intensities of the key ions obtained with various mass spectrometric techniques have been tabulated (Schuetzle & Jensen, 1985). Electron impact ionization, recording full spectra or selected ions (selective ion monitoring), is used widely, and more than 50 nitroarenes have been identified tentatively in extracts of diesel exhaust by high-resolution mass spectrometry (Xu *et al.*, 1982). More recently, chemical ionization was introduced into the analysis of nitroarenes, both as electron capture negative ion chemical ionization and as positive ion chemical ionization. A detection limit of 1 pg has been reported for 2-methyl-1-nitronaphthalene using negative ion chemical ionization (Ramdahl & Urdal, 1982).

Negative ion atmospheric pressure ionization mass spectrometry has also been applied to the analysis of nitroarenes and their metabolites, which, due to their high electron affinity, can be detected selectively by this technique; a good spectrum has been obtained with as little as 5 pg 1-nitropyrene (Korfmacher *et al.*, 1984, 1987, 1988). With this method, the limit of detection for 1-nitropyrene was 0.5 pg (Korfmacher & Miller, 1984) and that for 1-nitronaphthalene, 0.3 pg (Korfmacher & Rushing, 1986).

Triple-quadrupole mass spectrometry has been used to analyse nitroarenes in diesel exhaust, and the presence of various dinitroarenes was demonstrated, in addition to the commonly found mononitroarenes (Henderson *et al.*, 1983). Concentrations of dinitroarenes in diesel particulate extracts have been reported (Nishioka *et al.*, 1982; Schuetzle *et al.*, 1982).

In most studies, mass spectrometry has been used in combination with gas chromatography, but coupling with HPLC has also been reported (Levine *et al.*, 1982).

(d) Formation of nitroarenes during sample collection and loss during storage

Nitroarenes may be formed to some extent during sample collection by reaction of PAH with nitrogen oxides, and various experiments have been undertaken to estimate the extent of this effect (see the monograph on diesel and gasoline engine exhausts, p. 80).

Conversion of pyrene into nitropyrene and of mononitropyrene into dinitropyrenes during long-term absorption on silica has been reported (Hughes *et al.*, 1980). Nitroarene concentrations in diesel extracts have been found to decrease significantly during storage, whereas concentrations in particles were more stable (Nishioka *et al.*, 1982).

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 36 female weanling CD rats received oral intubations of 10 μmol [2.5 mg]/kg bw 1-nitropyrene (purity, >99.9%) in dimethyl sulfoxide (DMSO; 1.7 μmol [0.5 mg]/ml DMSO) three times per week for four weeks (average total dose, 16 μmol [4.7 mg]/rat) and were sacrificed after 76–78 weeks or when moribund (King, 1988). A group of 36 females received DMSO only. The number of 1-nitropyrene-treated rats with mammary tumours (16/35; five with adenocarcinomas, nine with fibroadenomas) was not different from controls (12/35). [The Working Group noted the short duration of both treatment and observation.]

Groups of 40, 40 and 46 female specific-pathogen-free Fischer 344/Jcl rats, six weeks old, received intragastric instillations of 5, 10 and 20 mg/kg bw, respectively, of 1-nitropyrene (impurities: 0.11% 1,3-dinitropyrene, 0.27% 1,6-dinitropyrene and 0.23% 1,8-dinitropyrene) in olive oil twice a week for 55 weeks (Odagiri *et al.*, 1986). A group of 30 vehicle control rats received olive oil alone. Animals were killed when moribund or after 104 weeks, at which time the experiment was terminated; only rats surviving beyond experimental week 46, when the first tumour was observed, were evaluated. Mammary adenocarcinomas were induced in a dose-dependent manner in the three treated groups (in 2/36; 12/39 — $p < 0.001$; and 14/45 — $p < 0.001$, respectively); no adenocarcinoma was observed in vehicle controls. Clitoral gland tumours, most of which were diagnosed as squamous-cell carcinomas, developed in a dose-dependent manner in treated rats, and the numbers of rats with tumours in the high-dose (12/45; 11 with squamous-cell carcinomas) and intermediate-dose (11/39; nine with squamous-cell carcinomas) groups was significantly ($p < 0.001$) greater than that in controls (one adenoma). In addition, more animals in

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

the treated groups had mononuclear-cell leukaemia (high-dose, 27/45; mid-dose, 22/39; and low-dose, 23/36; $p < 0.05$) than among vehicle controls (9/28). [The Working Group noted the presence of dinitropyrene impurities and could not ascertain their potential effect on the outcome of the experiment.]

(*b*) *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 1-nitropyrene (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). 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, 3/20 treated animals and 1/20 control animals had developed skin tumours (mainly papillomas). This difference was not statistically significant. [The Working Group noted the small number of animals used.]

In a study of initiating activity (Nesnow *et al.*, 1984), six groups of 39–40 male and 39–40 female SENCAR mice, seven weeks old, received a single dermal application of 0–3.0 mg 1-nitropyrene (purity, >99.5%) in 0.2 ml acetone; animals receiving 3.0 mg had two applications. A group of 40 males and 40 females received a single application of 0.05 mg benzo[*a*]pyrene and served as positive controls. One week after initiation, all mice received skin applications of 12-*O*-tetradecanoylphorbol 13-acetate in 0.2 ml acetone twice a week for 30 weeks. At the end of this period, no significant increase in the number of mice with skin papillomas was observed in the 1-nitropyrene-treated groups, although all mice in the benzo[*a*]pyrene-treated group that survived beyond week 31 developed skin papillomas.

(*c*) *Intratracheal instillation*

Hamster: A group of 34 male Syrian golden hamsters, eight weeks old, received intratracheal instillations of 2 mg 1-nitropyrene (purity, 98%; impurities: 0.008% 1,3-dinitropyrene, 0.6% 1,6-dinitropyrene plus 1,8-dinitropyrene, and 1.3% pyrene) suspended in 0.2 ml phosphate buffer solution once a week for 15 weeks (Yamamoto *et al.*, 1987). A further group received 2 mg benzo[*a*]pyrene and a vehicle control group of 19 animals received buffer solution alone. All hamsters in the 1-nitropyrene-treated and control groups had died within 663 and 684 days, respectively, following the initial instillation; after the 15 instillations, 24 and 16 animals in these groups, respectively, were still alive. Two lung adenomas were detected in 2/21 treated animals (the three others were cannibalized); in one animal, the adenoma co-existed with a squamous-cell papilloma in the trachea. No tumour was observed in the respiratory organs of control animals, but they occurred in 19/22 animals treated with benzo[*a*]pyrene.

(*d*) *Intrapulmonary administration*

Rat: A group of 32 male Fischer 344/DuCrj rats, 10-11 weeks old, received a single injection of 0.05 ml beeswax-tricaprylin containing 1.5 mg 1-nitropyrene (purity, >99.9%)

directly into the lower third of the left lung after left lateral thoracotomy (Maeda *et al.*, 1986). A group of 19 rats received a single injection of 0.05 ml beeswax-tricaprylin containing 0.5 mg 3-methylcholanthrene [purity unspecified], and another group of 31 rats received beeswax-tricaprylin only. Animals were observed for 72 weeks after treatment, at which time the experiment was terminated. No squamous-cell carcinoma of the lung was induced in rats injected with 1-nitropyrene or in vehicle controls, but all 19 rats injected with 3-methylcholanthrene developed these tumours. No difference in the incidence of tumours in other organs was observed among the three groups. [The Working Group noted the short period of observation.]

(*e*) *Subcutaneous administration*

Mouse: A group of 20 male BALB/c mice, six weeks old, received subcutaneous injections of 0.1 mg 1-nitropyrene (purity, >99.9%) dissolved in 0.2 ml DMSO once a week for 20 weeks (total dose, 2 mg; Tokiwa *et al.*, 1984). A group of 20 vehicle controls received injections of DMSO only. All animals were observed for 60 weeks or, for mice with tumours at the site of injection, until moribund. No subcutaneous tumour developed at the injection site in mice administered 1-nitropyrene or DMSO. In a group treated with the same dose of 1,6-dinitropyrene (see p. 219), 10/20 mice developed subcutaneous tumours. Lung tumours were found in 6/20 1-nitropyrene-treated and in 7/20 control mice. [The Working Group noted the small number of animals used and the short period of observation.]

Rat: A group of 20 male Fischer 344/DuCrj rats, eight weeks old, received subcutaneous injections of 2 mg 1-nitropyrene (purity, >99%) dissolved in 0.2 ml DMSO twice a week for ten weeks (Ohgaki *et al.*, 1982). A control group of 20 male rats received injections of 0.2 ml DMSO only. The animals were observed for life; the last rats died on day 377. The first tumour in the treated group was seen after 162 days; 8/17 of the animals surviving beyond this time developed tumours, described as one extraskeletal osteosarcoma and seven malignant fibrous histiocytomas at the site of injection. Two of the malignant histiocytomas proved to be serially transplantable into the subcutis of the same strain over 14 generations. No tumour was observed in controls ($p < 0.003$). [The Working Group noted that the authors reported in a later publication (Ohgaki *et al.*, 1985) that these findings were possibly due to contamination of the preparation of 1-nitropyrene with dinitropyrenes (about 0.8%) and not to 1-nitropyrene itself.]

A group of 20 male Fischer 344/DuCrj rats, six weeks old, received subcutaneous injections of 2 mg 1-nitropyrene (impurities: <0.05% each of 1,3-, 1,6 and 1,8-dinitropyrene, 1,3,6-trinitropyrene and 1,3,6,8-tetranitropyrene) dissolved in 0.2 ml DMSO twice a week for ten weeks (total dose, 40 mg); ten rats were treated with 0.2 mg 1-nitropyrene (total dose, 4 mg; Ohgaki *et al.*, 1985). A further group of 20 rats received injections of 0.2 ml DMSO only. Observation was terminated on day 650. No tumour was found at the site of injection in treated or control animals. Two groups treated with total doses of 0.4 mg 1,8-dinitropyrene (see p. 235) or 4 mg 1,6-dinitropyrene (see p. 219) all developed sarcomas. [The Working Group noted the small number of animals used and the short period of observation.]

A group of 31 male and 32 female newborn Sprague-Dawley-derived CD rats received subcutaneous injections of 100 μmol[25 mg]/kg bw 1-nitropyrene (<0.02% dinitropyrenes) dissolved in DMSO once a week for eight weeks (Hirose *et al.*, 1984). Another group of 29 males and 31 females received injections of 50 μmol[12.5 mg]/kg bw 1-nitropyrene in DMSO. A further group of 28 male and 31 female rats receiving DMSO only served as controls. The experiment was terminated when animals were 62 weeks old. In the group injected with the higher dose of 1-nitropyrene, 10/31 males and 9/32 females developed sarcomas, primarily malignant fibrous histiocytomas, at the site of injection. Of the females, 15/32 also had mammary tumours (ten adenocarcinomas, seven fibroadenomas). In the group given the lower dose, 2/29 males and 3/31 females developed tumours at the site of injection, and mammary tumours were found in 7/31 (three adenocarcinomas, five fibroadenomas) females. No tumour was detected at the site of injection in control animals, but mammary tumours were found in 2/31 females. There was a dose-response relationship for the induction of tumours at the site of injection, and the incidence of tumours in males ($p < 0.001$) and females ($p < 0.01$) in the group given the higher dose of 1-nitropyrene was significantly different from that in controls. The average period of induction for tumours at the injection site was shorter in males given the high dose (262 days) than in males given the low dose (312 days); this response was not observed in females (288 and 285 days). There was a dose-related increase in the formation of mammary gland tumours in treated females, and the incidence of mammary tumours in the high-dose group was significantly different from that in controls ($p < 0.001$). The numbers of mammary tumours (29 and nine), especially adenocarcinomas (16 and four), were also dose-related. Although some tumours were observed in other organs, the incidences were not different between treated and control animals.

A group of 49 female newborn CD rats received subcutaneous injections of 1-nitropyrene (purity, >99.9%) dissolved in DMSO (1.7 μmol [0.4 mg]/ml DMSO) into the suprascapular region once a week for eight weeks (total dose, 6.3 μmol [1.6 mg]; King, 1988). Another group of 40 animals received DMSO alone. Rats were observed until moribund or up to 67 weeks, at which time no malignant fibrous histiocytoma was found in either group. The number of rats with mammary tumours did not differ significantly between treated (16/49) and control animals (8/40), but a higher prevalence of adenocarcinoma-bearing animals was observed in the treated group. [The Working Group noted the low dose used and the short observation period.]

A group of 29 female weanling CD rats received subcutaneous injections of 100 μmol [25 mg]/kg bw 1-nitropyrene (purity, >99.9%) dissolved in DMSO (70 μmol[17 mg]/ml DMSO) once a week for five weeks (total dose, 77 μmol[19 mg]/rat; King, 1988). Another group of 30 rats received DMSO alone. Rats were observed until moribund or up to 88 weeks, at which time more rats in the treated group had mammary adenocarcinomas and fibroadenomas (17/29) than controls (11/30; $p < 0.08$). [The Working Group noted the high and variable spontaneous incidence of mammary tumours in these studies.]

Groups of 48 female newborn CD rats and 55 female newborn Fischer 344 rats received subcutaneous injections of 100 μmol[25 mg]/kg bw 1-nitropyrene (purity, >99.9%) dissolved in DMSO (70 μmol[17 mg]/ml DMSO) once a week for eight weeks (total dose,

63 μmol[15.5 mg]; King, 1988). Groups of 47 CD and 55 Fischer 344 rats were injected with DMSO. Animals were sacrificed at 86 weeks. Mammary gland tumours developed in all groups, but the incidences did not differ between the treated and control groups. Four Fischer 344 rats injected with 1-nitropyrene had leukaemia, and this malignancy did not occur in controls ($p < 0.05$). [The Working Group noted the high and variable spontaneous incidence of mammary tumours in the CD rats and the unusually low incidence of leukaemia in control Fischer 344 rats.]

(f) Intraperitoneal administration

Mouse: Three groups of 15, 15 and 16 male and 14, 14 and 12 female A/J mice, six to eight weeks old, received 17 intraperitoneal injections of 1-nitropyrene (purity, >99%, with no dinitropyrenes (El-Bayoumy & Hecht, 1983); total doses, 175, 525 and 1575 mg/kg bw, respectively) in 0.1 ml trioctanoin over a period of six weeks (El-Bayoumy *et al.*, 1984a). A group of 16 males and 16 females received injections of trioctanoin only. Mice were sacrificed 18 weeks after termination of the treatment at 24 weeks, and their lungs were examined. In the group given the highest dose of 1-nitropyrene, the number of male and female mice with lung tumours (22/28) was significantly higher ($p < 0.05$) than in controls (7/32); the mean number of lung tumours/mouse was also significantly increased (1.3 compared with 0.3 lung tumours/mouse; $p < 0.001$). The combined tumour incidences in the other two groups were not statistically different from that in controls, but the tumour incidence in males receiving the lowest dose was significantly greater (4/10). In each dose group, the numbers of mice with lung tumours and mean numbers of lung tumours/mouse were larger in males than in females. [The Working Group noted that studies conducted with strain A mice are usually considered to be of a screening nature and not definitive tests for carcinogenicity.]

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1-nitropyrene (purity, >99%; total doses, 700 or 2800 nmol [173 or 692 μg]) in 10, 20 and 40 μl DMSO on days 1, 8 and 15 after birth; 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, 34 males and 50 females given 700 nmol 1-nitropyrene, 29 males and 26 females given 2800 nmol 1-nitropyrene, 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 group were still alive. All remaining mice were killed after one year. Liver-cell tumours developed in 5/34 (two adenomas, three carcinomas) males treated with 700 nmol 1-nitropyrene and in 8/29 (three adenomas, five carcinomas) treated with 2800 nmol; the latter incidence was significantly greater than that in DMSO controls (2/28 and 5/45; $p < 0.05$). 1-Nitropyrene did not induce liver-cell tumours in females. The numbers of mice with lung tumours and with malignant lymphomas (1/29, 6/34) were not different from those in control mice. Benzo[*a*]pyrene induced liver-cell tumours in 18/37 males, but not in females. The numbers of benzo[*a*]pyrene-treated mice with lung tumours (males, 13/37; females, 13/27) were significantly greater than that in vehicle controls ($p < 0.005$). Of the vehicle controls, 2/28 and 5/45 males had liver tumours 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. [The Working Group noted the short observation period.]

Rat: A group of 36 female weanling CD rats received intraperitoneal injections of 10 μmol[2.5 mg]/kg bw 1-nitropyrene (purity, >99.9%) in DMSO (1.7 μmol[0.4 mg]/ml DMSO) three times per week for four weeks (total dose, 16 μmol (4 mg) per rat); 36 control animals received injections of DMSO only (King, 1988). Animals were sacrificed when moribund or after 76–78 weeks. Mammary tumours were found in 25/36 treated animals (14 adenocarcinomas, 19 fibroadenomas) and in 7/31 vehicle controls ($p < 0.0001$).

In a second study in the same laboratory (King, 1988), 29 female weanling CD rats received five weekly intraperitoneal injections of 100 μmol[25 mg]/kg bw 1-nitropyrene (purity, >99.9%) dissolved in DMSO (70 μmol[17 mg]/ml DMSO; total dose, 77 μmol[19 mg]/rat); 30 rats received DMSO alone. Animals were observed until moribund or up to 88 weeks. Mammary adenocarcinomas and fibroadenomas were observed in 17/29 treated rats and in 11/30 controls ($p < 0.08$). [The Working Group noted the inconsistent findings and the variations in the incidences of mammary tumours in controls.]

3.2 Other relevant data

(*a*) *Experimental systems*

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

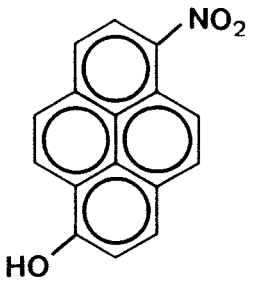
The kinetics and metabolism of 1-nitropyrene have been reviewed in recent articles on nitropyrenes (Beland *et al.*, 1985; Rosenkranz & Mermelstein, 1985; Rosenkranz & Howard, 1986; Tokiwa & Ohnishi, 1986). The major phase I metabolites identified are shown in Figure 3 (Beland *et al.*, 1985).

Studies in vivo

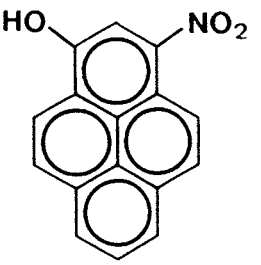
The principal metabolic pathways and metabolites in urine, faeces and bile have been identified in rats following oral, intravenous or intraperitoneal administration of radiolabelled 1-nitropyrene. Most administered 1-nitropyrene is accounted for by biliary excretion. For example, in one study on bile duct-cannulated rats, over 60% of the dose was excreted in bile over 24 h (Medinsky *et al.*, 1985). Most of this material is eventually excreted in the faeces, e.g., over 80% within 96 h (Ball, L.M. *et al.*, 1984a). Biliary metabolites have been characterized mainly as glucuronide and glutathione conjugates of oxidized nitropyrene metabolites (Howard *et al.*, 1985; Ohnishi *et al.*, 1986; Djurić *et al.*, 1989). Urinary metabolites are excreted in conjugated form, mainly with glucuronic acid (Ball, L.M. *et al.*, 1984a). In only one study in rats was excretion greater in urine than in faeces (Dutcher *et al.*, 1985).

Effects of gut microflora: The significance of gut microflora in the metabolism of 1-nitropyrene *in vivo* was demonstrated in several studies employing conventional (El-Bayoumy *et al.*, 1983; El-Bayoumy & Hecht, 1984; Kinouchi *et al.*, 1986b) and germ-free (El-Bayoumy *et al.*, 1984b; Kinouchi *et al.*, 1986b) or antibiotic-treated (Medinsky *et al.*, 1985) rats. Conventional but not germ-free or antibiotic-treated rats metabolized 1-nitropyrene to 1-aminopyrene.

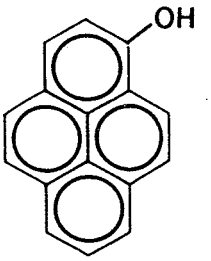
Fig. 3. Phase I metabolites of 1-nitropyrene[a]



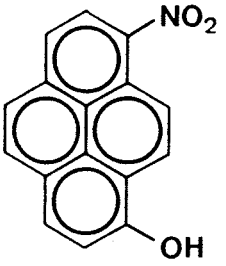
6-Hydroxy-1-nitropyrene



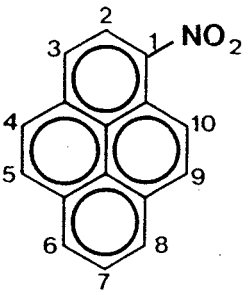
3-Hydroxy-1-nitropyrene



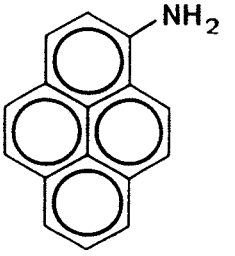
1-Hydroxypyrene



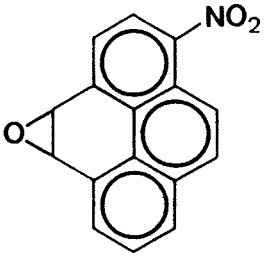
8-Hydroxy-1-nitropyrene



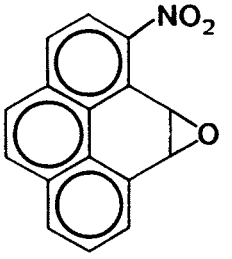
1-Nitropyrene



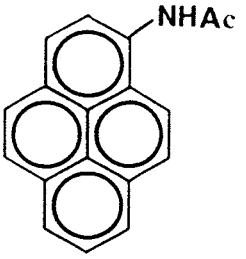
1-Aminopyrene



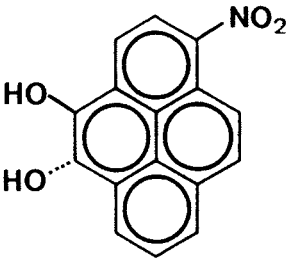
1-Nitropyrene-4,5-oxide



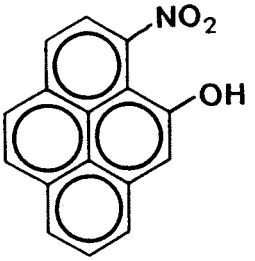
1-Nitropyrene-9,10-oxide



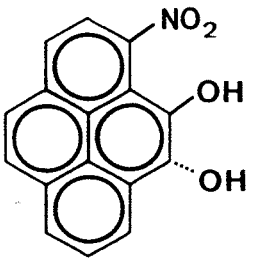
N-Acetyl-1-aminopyrene



1-Nitropyrene-*trans*-4,5-dihydrodiol



10-Hydroxy-1-nitropyrene



1-Nitropyrene-*trans*-9,10-dihydrodiol

[a]From Beland *et al.* (1985)

Effects of particle association: Groups of Fischer 344 rats were exposed to [^{3}H]1-nitropyrene by nose-only inhalation, either as a coating (about 6% by mass) on relatively insoluble, ultrafine ^{67}gallium oxide particles (6.2 mg/m^{3}) or as a homogeneous ultrafine aerosol (43 μg/m^{3}). Rats exposed to 1-nitropyrene on particles excreted the majority of the deposited radioactivity in the faeces (75 ± 18%), whereas animals exposed to 1-nitropyrene aerosol excreted a major portion of the radiolabel in the urine (76 ± 18%). There was no difference in the rates of lung clearance of 1-nitropyrene between the two groups. Most of the aerosol was cleared from the respiratory tract by direct absorption into the blood, while particle-associated nitropyrene was cleared by both blood absorption and mucociliary clearance followed by ingestion and faecal excretion (Sun *et al.*, 1983).

Male Fischer 344 rats were exposed by nose-only inhalation to various concentrations of [^{14}C]1-nitropyrene and [^{14}C]1-nitropyrene coated on diesel exhaust particles (50–1100 μg/m^{3} 1-nitropyrene; particulate concentration, 70–7200 μ/m^{3}). Over the range of concentrations tested, the pathways for excretion of [^{14}C]1-nitropyrene in urine and faeces were independent of the concentration of nitropyrene, whether given alone or associated with diesel exhaust particles. In all cases, faecal excretion was the major route of elimination, about twice as much being excreted by this route as in the urine. The fractional deposition of [^{14}C]1-nitropyrene in the respiratory tract did not appear to be dependent on the concentration. Half-times for elimination of ^{14}C in urine and faeces were about 15–20 h. Lungs of rats exposed to [^{14}C]1-nitropyrene coated on diesel exhaust particles contained nearly five times more ^{14}C than lungs from rats exposed to [^{14}C]-1-nitropyrene alone within 1 h after exposure. This difference was increased to 80-fold at 94 h after exposure. The long-term half-time for clearance of ^{14}C in the lungs of rats exposed to coated diesel particles was 36 days, in contrast to two days after exposure to 1-nitropyrene alone. The gastrointestinal absorption of the same 1-nitropyrene preparations was studied after an oral dose of 10 μg/kg bw. Within 1 h, >90% of ^{14}C was found in nitropyrene metabolites (Bond *et al.*, 1986).

[The Working Group noted that, on the basis of lung retention, 1-nitropyrene coated on gallium oxide is a poor model for 1-nitropyrene coated on diesel particles.]

The overall excretion pattern of ^{14}C was similar after intratracheal instillation of male Sprague-Dawley rats with [^{14}C]1-nitropyrene (8 nmol [2 μg]) either coated onto diesel particles (dose, 20 mg/kg bw), instilled along with unlabelled diesel particles, or administered alone (Ball *et al.*, 1986), and was also similar to that seen after intraperitoneal injection of [^{14}C]1-nitropyrene alone (Ball, L.M. *et al.*, 1984a). Lung retention was also similar to that following inhalation (described above). Protein-associated radioactivity has been observed in particle-treated lungs, with no detectable level of DNA adducts found up to 24 h after administration (Ball *et al.*, 1986).

DNA binding: DNA binding occurs in rat liver (Hsieh *et al.*, 1986) and in mouse lung (Mitchell, 1985a) after the administration of 1-nitropyrene. Less radioactivity was associated with lung macromolecules in antibiotic-treated rats than in controls (Ayres *et al.*, 1985). *N*-(Deoxyguanosin-8-yl)-1-aminopyrene has been identified in rat kidney, liver and mammary gland (Hashimoto & Shudo, 1985; Stanton *et al.*, 1985) and mouse lung (Mitchell, 1988); other unidentified adducts have been reported (Roy *et al.*, 1987; Mitchell,

1988). However, in another study, DNA was not bound in tissues of rats given 1-nitropyrene intraperitoneally (Djurić *et al.*, 1988).

Factors affecting metabolism: As reported in an abstract, newborn mice metabolized 1-nitropyrene more efficiently than older mice; the predominant metabolites were phenols and dihydrodiols (El-Bayoumy & Hecht, 1986).

Pretreatment with benzo[*a*]pyrene increased the radioactivity associated with DNA in the lungs of mice administered [^{14}C]1-nitropyrene (Mitchell, 1985a; Howard *et al.*, 1986); however, pretreatment with diesel extract had no effect (Howard *et al.*, 1986).

The capacity of liver microsomes to catalyse the oxidative metabolism of 1-nitropyrene was unchanged after rats were treated with 8 mg/kg bw 1-nitropyrene. Liver cytosolic and microsomal nitroreductase activities toward 1-nitropyrene were increased two-fold. DNA binding of 1-nitropyrene *in vitro* was two-fold higher in the presence of cytosol from 1-nitropyrene-pretreated rats (Djurić *et al.*, 1988).

Studies in vitro

Perfused organs: In isolated perfused and ventilated rat lungs, the major metabolites of [^{14}C]1-nitropyrene were 3-, 6-, and 8-hydroxy-1-nitropyrene; smaller quantities of 10-hydroxy-1-nitropyrene, 1-aminopyrene and *N*-acetyl-1-aminopyrene were also detected. Pretreatment with 3-methylcholanthrene increased the rate of metabolism ten-fold and the extent of radioactivity associated with tissue macromolecules 20-fold (Bond & Mauderly, 1984). Pretreatment of rats with diesel exhaust (particles, 7.4 mg/m^3) for four weeks increased the rate of metabolism in perfused lung and in nasal tissue two-fold and the extent of radioactivity associated with tissue macromolecules in the perfused lung four-fold (Bond *et al.*, 1985).

In isolated perfused rat livers, N-acetyl-1-aminopyrene was the major metabolite of [^{14}C]1-nitropyrene; smaller quantities of 1-aminopyrene and hydroxy-1-nitropyrenes were detected (Bond *et al.*, 1984).

Cultured cells: Chinese hamster ovary cells, Chinese hamster lung fibroblasts, calf thymus cells, rabbit alveolar macrophages, rabbit epithelial cells and human diploid fibroblasts catalysed the reduction of 1-nitropyrene to an intermediate which bound to DNA, giving an adduct identified as *N*-(deoxyguanosin-8-yl)-1-aminopyrene (Heflich *et al.*, 1985b; Jackson *et al.*, 1985; Beland *et al.*, 1986; Edwards *et al.*, 1986a; Heflich *et al.*, 1986a; Patton *et al.*, 1986; Gallagher *et al.*, 1988; Maher *et al.*, 1988). Incubation of rabbit lung and tracheal tissues with [^{14}C]1-nitropyrene resulted in association of the radioactivity with cellular DNA (King *et al.*, 1983).

Primary rat hepatocytes, Chinese hamster V79 cells and human hepatoma HepG2 cells catalysed the conversion of 1-nitropyrene into 1-aminopyrene (Salmeen *et al.*, 1983; Eddy *et al.*, 1987). Oxidized metabolites were also detected with the latter cell line (Eddy *et al.*, 1987).

Subcellular fractions: Cytosolic preparations from the livers of rats (Nachtman & Wei, 1982; Djurić *et al.*, 1985, 1986a, 1988), rabbits (Tatsumi *et al.*, 1986) and dogs (Djurić *et al.*, 1985) catalysed the reduction of 1-nitropyrene to 1-aminopyrene. Postmitochondrial supernatants of rat liver, lung and nasal tissue and of rabbit and hamster lung and liver catalysed

both the oxidation and reduction of 1-nitropyrene (Nachtman & Wei, 1982; Bond, 1983; El-Bayoumy & Hecht, 1983; Ball, L.M. *et al.*, 1984b; King *et al.*, 1984; Saito *et al.*, 1984a; Belisario *et al.*, 1986; Dybing *et al.*, 1986; Tatsumi *et al.*, 1986). Guinea-pig liver microsomes also catalysed the oxidation of 1-nitropyrene (Fifer *et al.*, 1986). In some instances, this metabolism was accompanied by binding to exogenous DNA (Ball & Lewtas, 1985; Djurić *et al.*, 1985, 1986b; Dybing *et al.*, 1986; Djurić *et al.*, 1988). Following incubation of [^{3}H]1-nitropyrene with calf thymus DNA, bovine xanthine oxidase and hypoxanthine at 37°C, covalent binding to DNA was shown to be proportional to the amount of reducing enzyme present (Howard & Beland, 1982).

Bacteria: Several strains of bacterial and gut microflora from animals and humans have been shown to reduce 1-nitropyrene (Kinouchi *et al.*, 1982; El-Bayoumy *et al.*, 1983; Howard *et al.*, 1983a; Cerniglia, 1985; Heflich *et al.*, 1985b; Manning *et al.*, 1986). In some instances, this metabolism was accompanied by the formation of a DNA adduct identified as *N*-(deoxyguanosin-8-yl)-1-aminopyrene.

(ii) *Toxic effects*

Groups of male and female specific-pathogen-free Fischer 344 rats that received single oral doses of up to 5 g/kg bw 1-nitropyrene as a fine powder suspension in 2% gelatin showed no mortality or histological damage in a wide range of organs examined when the animals were killed 4 or 14 days after administration (Marshall *et al.*, 1982).

Topical application and intraperitoneal administration of 1-nitropyrene to rats induced cutaneous and hepatic drug and carcinogen metabolism (Asokan *et al.*, 1985, 1986; Belisario *et al.*, 1988; Mukhtar *et al.*, 1988) and nitroreductase activity (Chou *et al.*, 1986; Djurić *et al.*, 1988).

Intraperitoneal injection of 1-nitropyrene (105 μmol[26 mg]/kg bw) into female Sprague-Dawley rats induced an oncofetal protein (Hanausek-Walaszek *et al.*, 1985). Superoxide radical was generated on incubation of rat lung microsomes with 1-nitropyrene (Nachtman, 1986).

(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).

[It is to be noted that, on occasion, 1-nitropyrene contains small quantities of dinitropyrenes (e.g., Odagiri *et al.*, 1986; Yamamoto *et al.*, 1987). Due to the potent mutagenicity of dinitropyrenes (Mermelstein *et al.*, 1981), their presence may affect the results. The Working Group has indicated in the text studies in which the purity of the compound tested was less than 99%.]

1-Nitropyrene induced DNA damage in *Escherichia coli* (at 0.5–2 μg/ml; Ohta *et al.*, 1984) and *Salmonella typhimurium* (lowest effective dose, 0.02 μg/ml; Nakamura *et al.*, 1987). It preferentially inhibited the growth of DNA repair-deficient *Bacillus subtilis* (at 0.2–1.0 μg/disc; Horikawa *et al.*, 1986).

1-Nitropyrene was mutagenic to *E. coli* WP2 *uvrA* pKM101 (Tokiwa *et al.*, 1984 (0.125–1 μg/plate); McCoy *et al.*, 1985a (0.3–33 μg/plate)) and to *S. typhimurium* TA96,

TA97, TA98, TA100, TA102, TA104, TA1537 and TA1538 (Rosenkranz *et al.*, 1980; Wang *et al.*, 1980; Löfroth, 1981; Mermelstein *et al.*, 1981; Pederson & Siak, 1981; Tokiwa *et al.*, 1981a, b; Pitts *et al.*, 1982; McCoy *et al.*, 1983a; Tokiwa *et al.*, 1984; Ball, L.M. *et al.*, 1984b; Heflich *et al.*, 1985a,b; McCoy *et al.*, 1985b; Rosenkranz *et al.*, 1985; Tokiwa *et al.*, 1985).

The urine of male rats receiving 10 mg/kg bw 1-nitropyrene intraperitoneally was mutagenic to *S. typhimurium* in the presence of β-glucuronidase and an exogenous metabolic system from rat liver (Ball, L.M. *et al.*, 1984a); the bile of treated rats was mutagenic in the presence and in the absence of an exogenous metabolic system (Morotomi *et al.*, 1985).

1-Nitropyrene (at up to 0.5 mg/ml) did not induce gene conversion or recombination in the yeast *Saccharomyces cerevisiae* D4 (McCoy *et al.*, 1983b, 1984).

1-Nitropyrene induced single-strand DNA breaks, as determined by alkaline elution, in primary mouse hepatocytes (at 10–200 μM; Møller & Thorgeirsson, 1985), in Chinese hamster DON lung fibroblasts (at 0.25–48 μg/ml; Edwards *et al.*, 1986b) and V79 cells (tested at 15 and 30 μM; Saito *et al.*, 1984b) and in cultured rat hepatoma cells (at 10–50 μM; Møller & Thorgeirsson, 1985).

1-Nitropyrene induced unscheduled DNA synthesis in cultured hepatocytes from mice (3.5×10^{-3}–3.5×10^{-2} mg/ml; Mori *et al.*, 1987), rats (Mori *et al.*, 1987 (3.5×10^{-3}–3.5×10^{-2} mg/ml); Kornbrust & Barfknecht, 1984 (5×10^{-7}–10^{-4} M, 97% pure)) and hamsters (Kornbrust & Barfknecht, 1984 (5×10^{-7}–10^{-4} M, 97% pure)). It was reported in an abstract to induce unscheduled DNA synthesis in human hepatocytes (Yoshimi *et al.*, 1987). It also induced unscheduled DNA synthesis in human (10^{-4} M; Sugimura & Takayama, 1983) and rat (10–100 μM; Doolittle & Butterworth, 1984) tracheal epithelial cells, in human hepatoma-derived HepG2 cells (Eddy *et al.*, 1986, 1987) and in rabbit lung Clara, but not alveolar type II, cells (Haugen *et al.*, 1986).

1-Nitropyrene preferentially killed DNA repair-deficient human xeroderma pigmentosum fibroblasts (Patton *et al.*, 1986 (20% survival at 25 μM); Maher *et al.*, 1988). This compound induced the synthesis of viral DNA in polyoma virus-transformed rat fibroblasts (at 10–30 μg/ml; Lambert & Weinstein, 1987).

1-Nitropyrene (at 33–60 μM) induced mutations at the 6-thioguanine locus of human diploid fibroblasts (Patton *et al.*, 1986; Maher *et al.*, 1988) and human hepatoma-derived HepG2 cells (at 2–20 μM; Eddy *et al.*, 1986, 1987) and had a marginal mutagenic effect on cultured Chinese hamster CHO cells (Marshall *et al.*, 1982 (at 2–20 μg/ml)) and V79 cells (Ball, J.C. *et al.*, 1984 (2–40.5 μM); Berry *et al.*, 1985 (only dose tested, 50 μM)), although no effect was observed in other studies with Chinese hamster CHO cells (Heflich *et al.*, 1985b, 1986a,b). The marginal effects were increased by the presence of an exogenous metabolic system from rat liver (Li & Dutcher, 1983 (20 μg/ml tested); Berry *et al.*, 1985 (50 μM tested)).

1-Nitropyrene (purity, 95%) was reported to be mutagenic to mouse lymphoma L5178Y cells at the $TK^{+/-}$ locus in the presence of an exogenous metabolic system (Lewtas, 1982). It did not induce mutation to diphtheria toxin resistance (at up to 20 μg/ml; Nakayasu *et al.*, 1982) or to ouabain resistance (at 1–10 μg/ml; Takayama *et al.*, 1983) in cultured Chinese hamster lung fibroblasts.

1-Nitropyrene (1–30 μM) induced sister chromatid exchange in cultured Chinese hamster CHO cells in the presence and absence of an exogenous metabolic system (Nachtman & Wolff, 1982) and was reported in an abstract to induce sister chromatid exchange in V79 cells (Heidemann & Miltenburger, 1983) and in CHO cells in the absence of an exogenous metabolic system (Lewtas, 1982; purity, 95%). It induced chromosomal aberrations, including chromosome and chromatid deletions and asymmetrical exchanges, in Chinese hamster DON lung fibroblasts (at 3.8–60 μg/ml; Lafi & Parry, 1987) and, as reported in an abstract, in Chinese hamster lung fibroblasts (Matsuoka *et al.*, 1987).

1-Nitropyrene (at 4–41 μM) induced morphological transformation in Syrian hamster embryo cells (DiPaolo *et al.*, 1983) and transformation (induction of growth in soft agar and invasiveness in chicken embryo skin cultures) in normal human fibroblasts (at 3–33 μM) under anaerobic conditions (Howard *et al.*, 1983b; Kumari *et al.*, 1984).

In mice, intratracheal instillation of 1-nitropyrene (at 10–100 mg/kg bw) induced damage in lung DNA as determined by alkaline elution (Mitchell, 1984, 1985a,b[abstract]; Mitchell, 1986 [abstract]).

Oral administration of 1-nitropyrene (at 0.5–5 g/kg bw) to rats induced a slight increase in the incidence of sister chromatid exchange in bone-marrow cells (Marshall *et al.*, 1982). It was reported in an abstract that increases in sister chromatid exchange and micronuclei frequency occurred in Chinese hamsters receiving 125 and 1000 mg/kg bw 1-nitropyrene, respectively (Heidemann & Miltenburger, 1983).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Epidemiological studies and case reports of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

1-Nitropyrene has been detected in some carbon blacks, in stack gases from coal-fired power plants and aluminium smelters and in particulate emissions from other stationary sources and from diesel and gasoline engines. 1-Nitropyrene also occurs at low concentrations in ambient air.

4.2 Experimental data[1]

1-Nitropyrene was tested for carcinogenicity by oral administration in rats, by skin application in mice, by intratracheal instillation in hamsters, by intrapulmonary administration in rats, by subcutaneous injection in mice and in newborn and young rats and by intraperitoneal injection in newborn and young mice and in rats. Two experiments by oral administration to rats were considered to be inadequate for evaluation. One experiment on mouse skin gave negative results; the other was considered to be inadequate. Following either intratracheal instillation in hamsters or intrapulmonary administration in rats, negative results were obtained.

One study by subcutaneous injection in young mice gave negative results, however the group was quite small. In one study in newborn rats, 1-nitropyrene produced sarcomas at the site of injection and an increased incidence of mammary tumours, including adenocarcinomas. In two other studies using newborn rats (including one using two different strains), no tumour was observed at the site of injection and there was no increase in the total number of mammary tumours. Two studies with young rats given subcutaneous injections of 1-nitropyrene yielded negative results, but the groups were small and the observation periods relatively short.

In a screening test by intraperitoneal injection using strain A mice, lung tumour incidence and the number of adenomas per mouse were significantly increased. One study using intraperitoneal injection in newborn mice showed an increase in the incidence of liver-cell tumours in males. One study on weanling rats showed an increased incidence of mammary tumours; a second study from the same laboratory showed a nonsignificant increase in the incidence of mammary tumours.

4.3 Human data

No data were available to the Working Group.

4.4 Other relevant data

The association of 1-nitropyrene with diesel particles led to a substantial reduction in clearance of the compound from the lungs of rats.

Metabolism of 1-nitropyrene led to DNA adduct formation in cultured human and mammalian cells and in animals. 1-Nitropyrene induced DNA damage and sister chromatid exchange in rodents; DNA damage, mutations and transformation in cultured human cells; and DNA damage, sister chromatid exchange, chromosomal aberrations, mutation and transformation in cultured animal cells. It was not recombinogenic to yeast but induced DNA damage and mutation in bacteria.

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[1]Subsequent to the meeting, the Secretariat became aware of a newly published study (El-Bayoumy *et al.*, 1988) describing the induction of mammary adenocarcinomas in female Sprague-Dawley rats given 1-nitropyrene (purity, >99.9%) by gavage from birth to 16 weeks of age.

Summary table of genetic and related effects of 1-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]		+[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 tables, 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

− considered to be negative

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity in experimental animals of 1-nitropyrene.

No data were available from studies in humans on the carcinogenicity of 1-nitropyrene.

Overall evaluation

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

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

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