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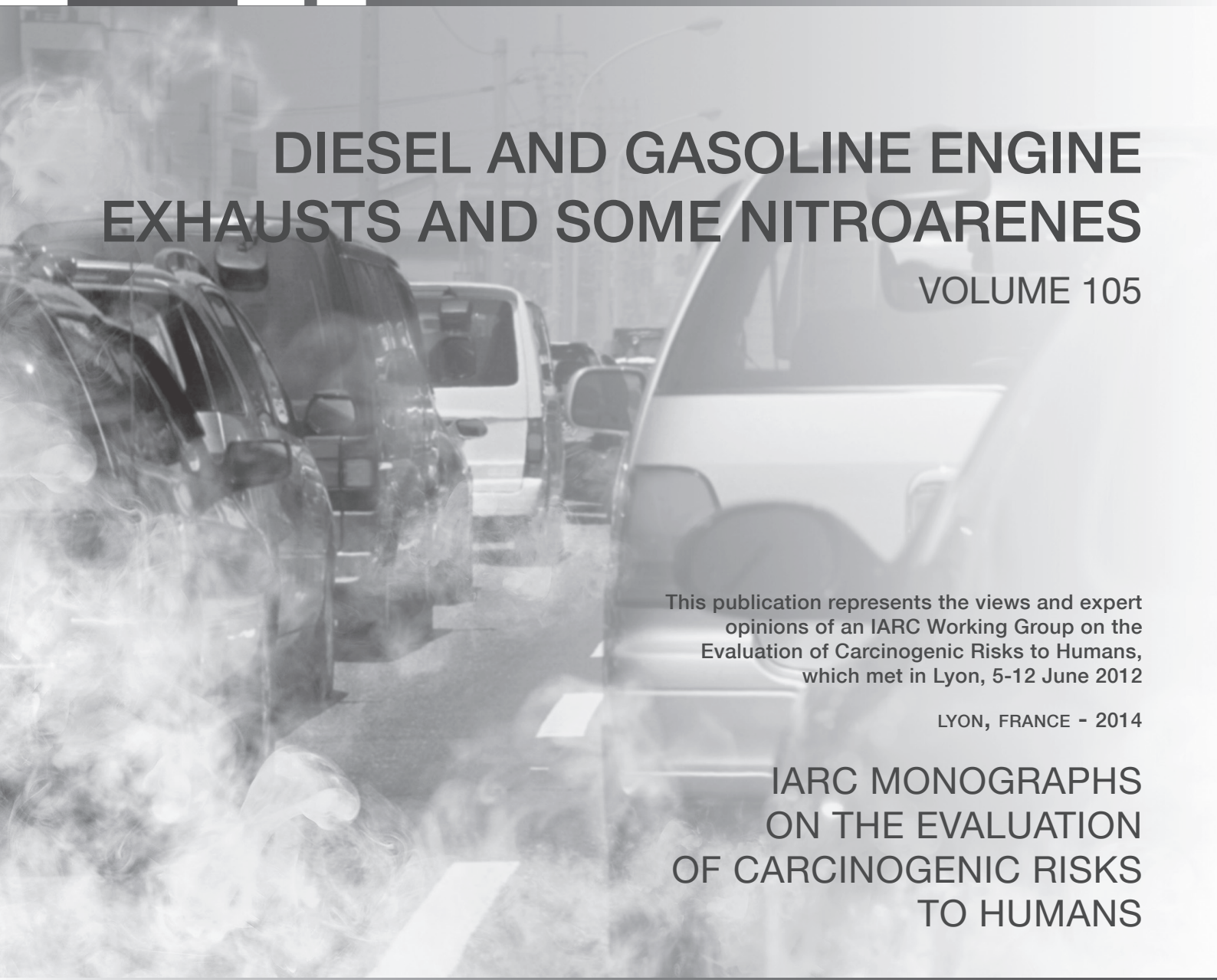

DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

1-NITROPYRENE

1-Nitropyrene was evaluated by a previous IARC Working Group in 1988 (IARC, 1989). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

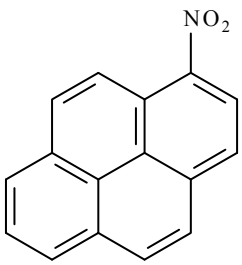
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 5522-43-0

IUPAC Name: 1-Nitropyrene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_9NO_2$

Relative molecular mass: 247.25

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow needles or prisms when crystallized from ethanol at room temperature (Luckenbach, 1980)

Melting-point: 155 °C (Luckenbach, 1980); 153 °C (Yaffe *et al.*, 2001)

Boiling-point: 472 °C at 101.3 kPa (Yaffe *et al.*, 2001)

Vapour pressure: 4.4×10^{-6} Pa at 20 °C (Yaffe *et al.*, 2001)

Octanol/water partition coefficient: Log $P_{o/w}$ 4.69 (Yaffe *et al.*, 2001)

Sorption coefficient: Log K_{oc} 4.48 (Yaffe *et al.*, 2001)

Henry's law constant: 6.4×10^{-2} kPa/m^3/g/mol at 25 °C (Yaffe *et al.*, 2001)

Spectroscopy data: Electron impact mass spectral data on ninefold deuterated 1-nitropyrene (1-nitro[$^2H_9$]pyrene) have been reported (Fatiadi & Hilpert, 1989).

Solubility: Soluble in water (0.017 mg/L; Yaffe *et al.*, 2001), ethanol, benzol and acetic acid (Luckenbach, 1980)

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, in the absence of air, 1-aminopyrene and 1-hydroxylaminopyrene (Boit *et al.*, 1965).

Stability: Readily decomposes on exposure to ultraviolet/visible light (Stärk *et al.*, 1985; Holloway *et al.*, 1987). Fan *et al.* (1996) reported half-lives of 0.8 hour for native and 0.5 hour for 1-nitro[$^{2}H_{9}$]pyrene adsorbed on diesel exhaust or wood smoke particles when exposed to sunlight in a smog chamber under ambient conditions.

1.1.4 Technical products and impurities

1-Nitropyrene is produced by the nitration of pyrene, the impurities of which are presumed to be dinitropyrenes. High-purity 1-nitropyrene is produced as a reference material for analytical determinations (see Section 1.3). 1-Nitro[$^{2}H_{9}$]pyrene of high isotopic purity was synthesized by Fatiadi & Hilpert (1989) and is commercially available at a purity of 98% from two companies in Germany and the USA. Native 1-nitropyrene and 1-nitro[$^{2}H_{9}$]pyrene are produced by several companies for use as an internal standard. Radiolabelled isotopes such as [^{14}C]- or [^{3}H]1-nitropyrene have been synthesized by research laboratories (el-Bayoumy & Hecht, 1984) or were acquired from commercial suppliers for use in animal studies.

1.2 Determination in air and analysis

1.2.1 Air sampling

1-Nitropyrene may be present at very low concentrations in the gas phase (Albinet *et al.*, 2006). To determine 1-nitropyrene adsorbed onto particles, a filter is used to collect the particles from diluted exhaust fumes or ambient air. During sampling, two types of artefact may occur: (a) conversion of the pyrene trapped on the filter surface to 1-nitropyrene and (b) conversion of the pyrene adsorbed onto particles to 1-nitropyrene (Grosjean *et al.*, 1983). This conversion was first observed on glass fibre membrane filters but not on polytetrafluoroethylene. To prevent the conversion of pyrene to 1-nitropyrene, pure polytetrafluorene (Teflon) or Teflon-coated filters can be used. Alternatively, 1-nitropyrene from diesel engine exhaust can be analysed in tailpipe deposits (Paschke *et al.*, 1992).

The amount of 1-nitropyrene adsorbed to the surface of diesel exhaust particles did not change after exposure to pure air, 100 ppb of ozone and 100 ppb of sulfur dioxide or 100 ppb of nitrogen dioxide (Grosjean *et al.*, 1983), suggesting that the influence of atmospheric conditions on the formation and stability of 1-nitropyrene is limited. However, when diesel exhaust particles are captured on a filter, the pyrene adsorbed on soot particles may still be nitrated (Butler & Crossley, 1981). The extent of the artificial formation of 1-nitropyrene was estimated to be below 20% when the duration of sampling engine exhaust in a dilution tunnel of a chassis dynamometer was less than 1 hour (Schuetzle, 1983; Hartung *et al.*, 1986; Lies *et al.*, 1986); however, due to the low temperatures and low concentrations of nitrating species, sampling of ambient air may last more than 4–8 hours (Dolan & Kittelson, 1979).

1.2.2 Analysis

More details on air sampling and the analysis of nitroarenes in general can be found in Section 1.2.2(d) of the *Monograph* on Diesel and Gasoline Engine Exhaust in this Volume.

To analyse 1-nitropyrene, three approaches have generally been applied: high-performance liquid chromatography (HPLC) with fluorescence detection, HPLC with chemiluminescence detection and gas chromatography (GC) or liquid chromatography with different types of mass spectrometry (MS)-based detection. Immunoaffinity methods have also been used with some success (Zühlke *et al.*, 1998).

An important step in the quantitative determination of 1-nitropyrene is extraction from the particles deposited on a filter. Acetone and

dichloromethane have been used to extract 1-nitropyrene from diesel exhaust particles, and sonication with acetone yielded quantitative extracts from standard reference material (SRM) 1650 (Jäger, 1978; Scheepers *et al.*, 1993), but good results were also obtained using other techniques such as supercritical fluid or Soxhlet extraction (Paschke *et al.*, 1992).

A small number of methods use the direct analysis of 1-nitropyrene by GC with electron capture (Jinhui & Lee, 2001), MS (Gorse *et al.*, 1983; Albinet *et al.*, 2006) or chemiluminescence detection (Hayakawa *et al.*, 1995). The National Institute of Occupational Safety and Health method 2560 is based on GC with chemiluminescence detection, with a working range of 0.020–50 µg and a sensitivity of 0.021–104 µg/m^3 for a 500-L sample, but is only suitable for samples with extremely high levels that are rarely encountered, even in indoor workplaces. More sensitive methods involve the reduction of the nitro group by bacterial nitroreductases or chemical nitroreduction. This step can be performed off-line using sodium hydrosulfide hydrate (Hisamatsu *et al.*, 1986) or online as part of the HPLC analysis using a zinc and glass granulate column (Scheepers *et al.*, 1993; Hayakawa *et al.*, 1996) or platinum/rhodium-coated aluminium (Murahashi *et al.*, 2003; Ohno *et al.*, 2009). The detection of 1-aminopyrene by fluorescence detection is highly sensitive, but that with chemiluminescence is more sensitive by approximately one order of magnitude (Murahashi *et al.*, 2003) and approaches the sensitivity accomplished by the use of MS. To enhance the sensitivity of detection using GC-MS, 1-aminopyrene is derivatized with heptafluoro butyric anhydride (Scheepers *et al.*, 1993). A small amount of deuterated 1-nitro[$^2H_9$]pyrene with high isotopic purity can be applied to the raw sample extract as an internal standard to adjust for the recovery in the reduction and derivatization steps. This analysis uses both retention time in capillary GC and the MS/MS spectrum to verify the identity of and was able to determine the lowest concentrations of 1-nitropyrene detected in airborne particulate matter (PM) to date (> 1 pg/m^3) (Scheepers *et al.*, 1993; Albinet *et al.*, 2006; Miller-Schulze *et al.*, 2007). Recently, a deuterated internal standard was also used in an HPLC analysis with fluorescence detection (Ohno *et al.*, 2009).

Certified SRMs for 1-nitropyrene are available from the National Institute of Standards and Technology (NIST) in Gaithersburg, USA: SRM 1648 (atmospheric PM collected in St. Louis, MO, in 1978; NIST, 1998), SRM 1649a (atmospheric PM collected in an urban area in Washington DC in the late 1970s; NIST, 2001), SRM 1650b (diesel exhaust PM collected from a heat exchanger; NIST, 2006) and SRM 2975 (industrial forklift; NIST, 2000). Albinet *et al.* (2006) reported values of 1-nitropyrene for each of these SRMs based on liquid chromatography-MS/MS analysis.

1.3 Production and use

The synthesis of 1-nitropyrene by heating pyrene with nitric acid and water was first reported by Graebe in 1871 (IARC, 1984). 1-Nitropyrene was also produced in a mixture with dinitropyrenes following the addition of potassium nitrite to pyrene in diethyl ether (Prager & Jacobson, 1922). More recently, Boit *et al.* (1965) described its synthesis by heating pyrene in nitric acid and glacial acetic acid. At present, 1-nitropyrene of up to 99% purity is produced in small amounts for use as a reference standard in chemical analysis, and is also available as a certified reference material (BCR305; 99% pure). Deuterated 1-nitro[$^2H_9$]pyrene is produced for use as an internal standard, and tritium-labelled 1-nitropyrene is produced for use in animal studies.

1.4 Occurrence

1.4.1 Diesel and gasoline exhaust emissions

1-Nitropyrene is the most abundant nitroarene in diesel engine emissions (Bamford *et al.*, 2003), and its formation is facilitated by the high temperatures and excess air supply in the combustion chamber of diesel engines, where it is generated by the addition of nitrogen oxide or nitrogen dioxide to free pyrene radicals (carbonium ions) (Schuetzle & Perez, 1983). Du & Kittelson (1984) observed a fourfold increase in 1-nitropyrene from the cylinder to the exhaust manifold, and Olah *et al.* (1981) also suggested that polycyclic aromatic hydrocarbons (PAHs) reacted with nitric acid in the exhaust manifold. When a diesel engine was run on nitrogen-free air, the amount of 1-nitropyrene produced was much lower (Herr *et al.*, 1982). The addition of pyrene to a pure *n*-hexadecane fuel resulted in increased emissions of 1-nitropyrene and other nitroarenes (Henderson *et al.*, 1984). Concentrations of 1-nitropyrene measured in emissions from vehicles that were placed on a chassis dynamometer are presented in Table 1.1, and showed wide variation, depending on the type of engine, the make of car and the driving cycle used. Passenger cars tended to have higher emissions of 1-nitropyrene during driving cycles when the engine was hot compared with those when the engine was cold (at the start of a driving pattern) (Scheepers *et al.*, 2001).

It has been suggested that the formation of 1-nitropyrene is dependent on the production of nitrogen dioxide in the combustion chamber, which is normally generated at much higher levels under elevated combustion temperatures. However, the formation of soot and PAHs tends to decrease at higher speeds (Steenlage & Rijkeboer, 1985), and the formation of 1-nitropyrene is therefore difficult to predict. Moreover, engines that have an indirect fuel injection system, which are used in light-duty vehicles such as passenger cars, small power generators and forklifts, yield somewhat higher levels of 1-nitropyrene in diesel exhaust particles by weight than direct-injection engines with which heavy-duty vehicles are usually equipped (Table 1.1; Yamaki *et al.*, 1986). 1-Nitropyrene was found in the vicinity of heavy-duty road traffic (heavy goods vehicles [HGVs] and buses), the platforms of airports (push-back tractors, tanker HGVs and luggage transport trains), railway tracks (diesel trains used for surface operations and underground mining), military or agricultural vehicles and excavation in the building and construction industry (surface and underground operations) (Scheepers *et al.*, 1993, 1994a, b, 1995a). Diesel-powered engines are also used in shipping, but few data were available on the emissions of ships' engines (Scheepers *et al.*, 1995a).

Several studies have measured the levels of 1-nitropyrene in emissions from diesel-powered road and off-road vehicles, such as forklifts, electric power supplies and lawn mowers (Table 1.2). Some earlier studies (Salmeen *et al.*, 1982; Schuetzle *et al.*, 1982) [not presented in Table 1.2] reported levels of 1-nitropyrene in emissions from light-duty passenger cars (mean per vehicle tested) in the range of 3.9–14.2 μg/g of total suspended fraction. Gibson (1982) reported levels of 55–2280 μg/g of soluble organic extract of 1-nitropyrene in emissions from light-duty passenger car models from 1978 to 1982.

Different types of current passenger car that were run on commercial fuel under conditions of the California unified driving cycle were tested on a dynamometer, together with used crankcase oil 'as received' (Zielinska *et al.*, 2004); some influence of the year of the model of car and testing conditions was observed.

The use of biodiesel and an oxidation catalyst was studied (Sharp *et al.*, 2000) using the heavy-duty transient Federal Test Procedure (FTP). In three separate heavy-duty diesel engines (one 1995 and two 1997 models), different fuels were used: neat biodiesel fuel (B100), a blend of biodiesel and normal diesel fuel 20:80 by

Table 1.1 1-Nitropyrene in exhaust emissions generated by simulated driving cycles on a chassis dynamometer

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Gasoline-powered engines						
Gibson (1982)	4.3-L 8-cylinder car with its catalyst removed using unleaded gasoline	23-min hot-start portion of the FTP	3	1981	4.3 ± 3.2	0.06 ± 0.06[a]
	2.5-L 4-cylinder catalyst car	23-min hot-start portion of the FTP	2	1980	0.63 ± 052	0.029[a]
	5.7 -L 8-cylinder precatalyst car using leaded gasoline	23-minute hot-start portion of the Federal Test Procedure	2	1974	3.9 ± 1.3	0.11 ± 0.033
Zielinska *et al.* (2004)	Mazda Millenia, Ford Explorer, Nissan Maxima, GMC 1500 Pickup, Mercury Sable	*Normal PM emitters*				
		California United Driving Cycle at 72 F	18	1982–96		0.016[a]
		California United Driving Cycle at 30 F	12	1992–96		0.03[a]
	Ford F-150 pick-up	Gasoline black smoker; California United Driving Cycle at 72 F	5	1976		0.19[a]
	Mitsubishi Montero	Gasoline white smoker; California United Driving Cycle at 72 F	2	1990		0.13[a]
Light-duty diesel-powered engines						
Gibson *et al.* (1981)	Oldsmobile	FTP	1	1978	3.9	–
			1	1978	8.2	–
Gibson (1982)	General motors 5.7-L 8-cylinder production model	23-min hot-start portion of the FTP	4	1980	8.0 ± 2.4	2.0 ± 0.75[a]
	General motors 5.7-L diesel car equipped with an experimental tube-type trap coated with ceramic fibres	23-min hot-start portion of the FTP	2	1980	14.2	0.75[a]
Gibson (1983)	Oldsmobile	FTP	1	1982	24.5	–
			1	1982	7.6	–
	Opel	FTP	1	1978	3.9	
Gorse *et al.* (1983)	Not specified	FTP	2	–		4.6
		Highway Fuel Economy Test	2	–		4.2
Scheepers *et al.* (1994a)	Not specified	European Driving Cycle (cold start)	1	1990		0.32
		European Driving Cycle (hot start)	1	1990		0.22
		US'75 Driving Cycle	1			0.36

Table 1.1 (continued)

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Scheepers *et al.* (2001)	French make (Citroen, Peugeot, Renault)	Urban Driving Cycle	7	1996	2.2 ± 0.6	–
		Urban Driving Cycle (hot start)	7	1996	5.1 ± 1.5	
		Extra-urban driving cycle	7	1996	10.4 ± 4.9	–
	German make (Opel)	Urban Driving Cycle	4	1996	2.5 ± 0.6	–
		Urban Driving Cycle (hot start)	4	1996	3.2 ± 0.8	–
		Extra-urban driving cycle	4	2001	7.4 ± 4.9	–
	Japanese make (Mazda)	Urban Driving Cycle	5	2001	21.2 ± 1.2	–
		Urban Driving Cycle (hot start)	5	2001	21.4 ± 6.9	–
		Extra-urban driving cycle	5	2001	91.4 ± 36.8	–
	American make (Chrysler)	Urban Driving Cycle	1	2001	6.7	
		Urban Driving Cycle (hot start)	1	2001	8.1	–
		Extra-urban driving cycle	1	2001	33.5	–
	German make (Mercedes)	FTP – cold transition period (0–505 s)	3	2001	6.0 ± 3.1	–
		FTP – stabilized period (505–1372 s)	3	2001	16.7 ± 9.1	–
		FTP – hot transition period (1372–1877 s)	3	2001	28.2 ± 4.7	–
	Japanese make (Nissan)	FTP – cold transition period (0–505 s)	5	2001	28.4 ± 15.8	–
		FTP – stabilized period (505–1372 s)	5	2001	18.5 ± 10.1	–
		FTP – hot transition period (1372–1877 s)	5	2001	44.9 ± 22.9	–
Bamford *et al.*(2003)	Diesel engine exhaust from industrial forklift (SRM2975)	Not specified	3	–	39.64 ± 1.7	–
	Diesel engine exhaust PM (SRM1975)	Not specified	3		16.07 ± 0.59	–
	Diesel engine exhaust PM (SRM1650a)	Collected from a heat exchanger	3	-	18.33 ± 0.34	–
Zielinska *et al.* (2004)	Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	Current technology diesel engine; California United Driving Cycle at 72 F	9	1998–2000		3.13[a]
	Dodge Ram 2500 Pickup	High PM emitter; California United Driving Cycle at 72 F	6	1991		1.77[a]
	Dodge Ram 2500 Pickup, Mercedes Benz E300, Volkswagen Beetle TDI	Current technology diesel engine; California United Driving Cycle at 30 F	6	1998–2000		6.21[a]

Table 1.1 (continued)

Reference	Type of car	Driving cycle or pattern	No. of samples or of driving cycles	Year	1-NP (AM ± SD)	
					µg/g	µg/km
Heavy-duty diesel-powered engines						
Draper (1986)	Mining engine	100% load, 1200 rpm	1	–	< 12	–
		75% load, 1800 rpm	1	–	5.0	–
Scheepers *et al.* (1994a)	Not reported	Suburban	1	1990	1.92	2.13
			1	1990	1.94	3.28
		Urban	1	1990	3.39	6.48
		Motorway	1	1990	1.94	0.87
			1	1990	2.73	1.37
Westerholm *et al.* (2001)	Volvo FH12 truck with D12A 420 diesel engine equipped with turbo, intercooler and electronic fuel-injection system (complies with WHO Regional Office for Europe 2 requirements)	Transient driving cycle for buses; engine fuelled with EPEFE reference fuel CEC-RF-73-A93	4	–	-	0.026 ± 0.04
		Transient driving cycle for buses; engine fuelled with a Swedish Environmental Classified diesel fuel (MK1)	4	–	–	< 0.005

[a] Converted from µg/mile by the Working Group

AM, arithmetic mean; EPEFE, European Programme on Emissions, Fuels and Engine Technologies; FTP, Test Procedure; 1-NP, 1-nitropyrene; PM, particulate matter; rpm, revolutions per minute; SD, standard deviation; SRM, standard reference material; TDI, Turbocharged Direct Injection; WHO, World Health Organization

Table 1.2 Concentrations of 1-nitropyrene in particulate matter emitted from diesel-powered engines at fixed locations

Reference	Description of source	Location and conditions	Year	No. of samples	1-NP (μg/g)		
					Respirable dust (μg/g)	Total suspended particles (μg/g)	Soluble organic fraction (μg/g)
Scheepers *et al.* (1994a)	Forklift truck in concrete production plant	Indoor; Netherlands	1992	2	1.9; 4.2	3.2 ± 2.8 (n = 12)	–
	Forklift truck in chemical plant	Indoor; Netherlands	1992	2	5.6; 7.0		
	Forklift truck in aluminium rolling	Indoor; Netherlands	1992	2	5.4; 7.7		
	Ship's aggregate in river vessel	Outdoo; Netherlands; wind speed 8–9 m/s	1992	1	9.1	7.6	11.9
	Lawn mowers in gardening	Outdoor; Netherlands; wind speed 5 m/s	1992	1		0.099	0.33
Rappaport *et al.* (1982)	Long distance road truck	4-stroke; 6-cylinder engine	1979–80	6	–	–	< 2 – 44
Nakagawa *et al.* (1983)	Bus	Isuzu BY30 at 1200 rpm (idle)	1970	1	70.5	30[a]	–
Scheepers *et al.* (1994a)	Train engine	Repair shop for diesel-powered trains; engines entered the workshop with a cold engine in the morning and exhaust was emitted during engine test runs; exhaust scavenging system did not have sufficient capacity.	1992	3	6.6 ± 1.6	1.3 ± 0.8	6.0 ± 2.6
			1994	3	–	7.4 ± 1.9[a]	–
	Ship's engine	Outdoor; Netherlands; wind speed 8–9 m/s	1992	3	–	0.97 ± 0.57	2.0 ± 0.94
	Airport platform vehicle	Outdoor; Netherlands; wind speed 6–13 m/s	1992	3	–	0.60 ± 0.23	1.4 ± 0.45
	Armoured cars	Military driving lessons facility			0.11 ± 0.0014	0.40 ± 0.025	
Yamazaki *et al.* (2000)	2.8-L diesel engine	Sample collected from the tailpipe of an idling engine	1993				4.3 pmol/mg
	2.5-L diesel engine		1996				29 pmol/mg
	7.4-L diesel engine		1989				63 pmol/mg

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

1-NP, 1-nitropyrene; rpm, revolutions per minute

Table 1.3 Emissions from heavy-duty vehicles with alternative fuels, particle filter and catalyst

Vehicle (engine)	Substance	B100 (100% biofuel[a]) (in ng/hp-h)			B20 (20% biofuel[a] in conventional diesel)		2D (100% conventional diesel fuel)	
Oxidation catalyst		–	–	+	–	+	–	+
Urban transit bus (DDC Series 50, 205 kW, n = 1)[b]	2-Nitrofluorene	48	40	14	70	67	88	90
	1-Nitropyrene	8.5	5.2	37	19	249	83	76
	6-Nitrochrysene	< 0.5	ND	1.8	0.8	4.0	0.8	5.8
Full-size pick-up truck (Cummins B5.9, 119 kW, n = 1)[c]	2-Nitrofluorene	142	122	73	–	365	257	478
	1-Nitropyrene	34	20	325	265	1644	210	2171
	6-Nitrochrysene	< 0.5	< 0.5	6.2	1.9	58	11	56

[a] Methyl ester from virgin soya bean oil (AG Environment Products)
[b] Low sulfur #2 diesel fuel (Chevron Phillips) and engine equipped with exhaust gas recirculation system for reduction of nitrogen oxides
[c] Ultra-low sulfur certified diesel fuel (Chevron Phillips) and engine equipped with exhaust gas recirculation system for reduction of nitrogen oxides, a crankcase emissions coalescer for nitrogen oxides and reduction of particulate matter and a two-stage particle filter consisting of a ceramic flow-through monolith diesel oxidation catalyst and a wall-flow monolithic catalysed soot filter
hp, horse power; ND, not detected
From Sharp *et al.* (2000)

volume (B20) and neat diesel fuel (2D). In the exhaust from B100, the formation of 1-nitropyrene was between 4.7 and 34 ng/horse power (hp)–h in the three engines (see Table 1.3). The addition of an oxidation catalyst increased the emission of 1-nitropyrene from one of the three engines to 325 ng/hp-h. One of the 1997 model engines run on B20 and 2D produced 19 and 83 ng/hp-h of 1-nitropyrene, respectively. The addition of a catalyst increased the values to 249 and 76 ng/hp-h for B20 and 2D, respectively. The 1995 model engine produced levels of 265 and 210 ng/hp-h of 1-nitropyrene with B20 and 2D, respectively, which increased to 1644 and 2171 ng/hp-h, respectively, after the addition of an oxidation catalyst.

Liu *et al.* (2010) compared two similar 15-L heavy-duty engines [not further specified] in an FTP cycle: a 2004 model equipped with a system to reduce nitrogen oxides and a 2007 model fitted with a reduction system for nitrogen oxides, a crankcase emission reducer of nitrogen oxides and a two-stage particle filter (diesel exhaust catalyst and catalysed soot filter). The level of 1-nitropyrene emitted from the 2004 model engine was 55 ± 6.44 ng/hp-h but was below the limit of quantification (0.25 ng/hp-h) in the 2007 model engine.

1.4.2 Exhaust fumes from gasoline-powered cars

Gasoline-powered spark ignition engines are less liable to emit nitroarenes than diesel engines. Alsberg *et al.* (1985) analysed different fractions of PAHs, including the polar fractions, but did not report 1-nitropyrene as a constituent in the extracts of particulate fractions of exhausts from gasoline-powered cars. Murahashi *et al.* (2003) reported a single measurement of 1-nitropyrene in the exhaust from a gasoline-powered vehicle [type of vehicle and driving cycle unspecified]. The emission of particles was low (0.01 g/km) and the amount of 1-nitropyrene was reported to be 0.02 µg/km compared with an [unspecified] diesel-powered vehicle that emitted 3.0 µg/km of 1-nitropyrene (also a single observation with a particle emission of 0.36 g/km). Gorse *et al.* (1983) determined that the on-road emission of 1-nitropyrene was < 0.03 µg/km in the Allegheny Mountain Tunnel, PA, USA, for predominantly light-duty gasoline passenger cars (of which 74% were estimated to be equipped with catalysts).

1.4.3 Aircraft exhaust

McCartney *et al.* (1986) observed elevated mutagenic activity in extracts from PM collected close to the runway of an airport, suggesting that nitrated mutagens are emitted by airplanes. [The Working Group noted that no data of chemical analysis were available to support this statement, and it was also not clear to what extent road traffic at the airport, remote sources of traffic or nitroarenes derived from atmospheric photochemistry may have contributed to the observed mutagenicity.]

1.4.4 Liquefied petroleum gas, gas burners and kerosene heaters

1-Nitropyrene, and 1,6- and 1,8-dinitropyrene in fumes produced by burning propane (or a mixture of gases including methane), using a Bunsen burner, co-eluted with standards in GC frame thermionic detection (Tokiwa *et al.*, 1985). A strong mutagenic response in the *Salmonella typhimurium* TA97 assay in the absence of an exogenous metabolic activation system was observed in the fraction that containing the reported nitroarenes. However, 1-nitropyrene was not detected in these samples [limit of detection not reported], or observed in kerosene heaters. In contrast, Kinouchi *et al.* (1988) detected 1-nitropyrene in the emissions from kerosene heaters using HPLC with fluorescence detection (see Table 1.4).

1.4.5 Emissions from industrial processes

Following the observation of elevated mutagenicity of extracts of carbon black-based photocopy toners (Löfroth *et al.*, 1980), 1-nitropyrene was found as a trace contaminant in toners, copiers and furnace carbon black produced before 1980 (Rosenkranz *et al.*, 1980; Ramdahl & Urdal, 1982). This was apparently the result of the production process used in 1967, which involved an oxidation step that resulted in the nitration of pyrene. After this discovery, the content of 1-nitropyrene was reduced from 5–100 µg/g to > 0.3 µg/g (Rosenkranz *et al.*, 1980; Sanders, 1981).

1-Nitropyrene was purported to be formed by waste incinerators (Gibson, 1982) and has been identified in coal fly ash (Harris *et al.*, 1984). Indirect evidence for the presence of nitroarenes, including 1-nitropyrene, was provided by the positive results of extracts from the emissions of a municipal-waste incinerator in the *Salmonella* mutagenicity assay (DeMarini *et al.*, 1996). Williams *et al.* (1986) did not detect 1-nitropyrene in roofing tar or remains from a coke oven, while another study reported levels of 27 µg/g of 1-nitropyrene in extracts of coke oven emissions (Topinka *et al.*, 1998).

1.4.6 Crankcase oils and wastewater

1-Nitropyrene was detected in crankcase oil at extremely high levels (138 µg/L of oil) and in water from oil–water separating tanks in gasoline stations at a range of < 0.00025–25.6 µg/L (Manabe *et al.*, 1984).

1.4.7 Surface water

1-Nitropyrene was detected at very low levels in river and seawater (Murahashi *et al.*, 2001). The highest values were observed in wet precipitations and were attributed to airborne particulates (see Table 1.4).

1.4.8 Food contamination and preparation

Table 1.5 summarizes levels of 1-nitropyrene in a variety of dietary products. The highest values were reported for products that may be contaminated by the deposition of particulates in outdoor air pollution (spices and different types of tea), through food preparation, such as the grilling of fish or meat, or during growing and further treatment (e.g. roasted tea) (Kinouchi *et al.*, 1986; Ohnishi *et al.*, 1986; Schlemitz & Pfannhauser, 1996a, b). High concentrations of 1-nitropyrene were found in fumes from different cooking oils at a range of 0.9–3.4 µg/m^3 (Table 1.4; Wu *et al.*,

Table 1.4 Concentrations of 1-nitropyrene in air/particulate matter and water from non-diesel sources

Reference	Source	Method of sampling and conditions	Method of analysis	No. of samples	1-NP (µg/g extracted PM)	1-NP (ng/m³)
Gibson (1982)	Wood fire smoke (burning of red oak in a fire place)	25-fold diluted flue gas sampled with a high volume sampler, collecting an unspecified fraction of PM	HPLC-fluorescence according to Gibson *et al.* (1981)	2	0.09 0.012	–
Thrane & Stray (1986)	Aluminium reduction plant	Unspecified fraction of particles collected on glassfibre filters by high-volume sampling in the potroom of a Söderberg electrode aluminium reduction plant	GC-MS analysis using a negative ion chemical ionization MS	1	–	64
Topinka *et al.* (1998)	Coke oven emissions	Total suspended particles collected on the top-side of the coke oven battery by high volume sampling on PTFE coated glassfibre filters	GC-MS analysis according to Scheepers *et al.* (1994a)	1	27	–
Taga *et al.* (2005)	Emissions from coal burning	Unspecified fraction of particle collected from the chimney of a domestic coal stove on a glassfibre filter	HPLC analysis with chemiluminescence detection	1	[240]	–
Kinouchi *et al.* (1988)	Indoor use of kerosene heater	Continuous sampling for 8 h	HPLC analysis with nitroreduction using enzymatic nitroreductase and fluorescence detection according to Manabe *et al.* (1984)	1	–	0.147
		21 samples of 20 min each at the beginning of burning with intermittent intervals of 20 min during which the room was ventilated		1	–	1.62
		Continuous sampling for 7 h with a latency of 1 h after lighting the heater during which the room was ventilated		1	–	0.044
Tokiwa *et al.* (1985)	Kerosene heater	Unspecified fraction of particles collected on XAD-2 resin for 2 h at an air flow rate of 20 L/min	Co-elution with standards in a GC analysis with frame thermionic detection according to Møller & Alfheim (1983)	2	ND	
	Gas[a] burned in a Bunsen burner			2	20.6	
	LPG (almost entirely propane) burned in a Bunsen burner			1	1.88	

Table 1.4 (continued)

Reference	Source	Method of sampling and conditions	Method of analysis	No. of samples	1-NP (µg/g extracted PM)	1-NP (ng/m³)
Murahashi *et al.* (2001)	River water	Midstream from Asano River, Suzumi, Kanazawa, Japan	HPLC with chemiluminescence detection according to Murahashi & Hayakawa (1997)	5		*pg/L* 1 2 3 5 27
	Seawater	Collected at seashore of the Sea of Japan at Kanaiwa, Kanazawa, Japan		2		0.2 0.5
	Precipitation (rain)	Collected on roof top in residential area of Kanazawa, Japan		2		370 3200

[a] 4–20% methane, 40–45% hydrogen, 10% carbon monoxide, 3–4% oxygen, 5–25% nitrogen, 3–4% butane and traces of pentane, isopentane and propane

GC, gas chromatography; HPLC, high-performance liquid chromatography; LPG, liquefied petroleum gas; min, minute; MS, mass spectrometry; 1-NP, 1-nitropyrene; ND, not detected; PM, particulate matter; PTFE, polytetrafluoroethylene; XAD, polymeric resin

Table 1.5 Occurrence of 1-nitropyrene in food stuffs and beverages

Reference	Category	Food stuff or beverage	No. of samples	1-NP (µg/g)
Schlemitz & Pfannhauser (1996a)	Vegetables and nuts	Lettuce	–	< 0.2
		Parsley	–	1.7
		Carrot	–	0.4
		Peanuts	–	< 0.5
	Spices and herbs	Paprika	–	9.3
		Marjoram	–	14.1
		Caraway	–	10.9
Spitzer *et al.* (2000)	Herbs	Basil	1	0.0014
		Chervil	1	0.0001
		Marjoram	2	0.0002; 0.0004
		Oregano	1	0.0014
		Sage	1	0.0001
Wu *et al.* (1998)	Cooking oils	Lard oil	3	1.1 ± 0.1[a]
		Soya bean oil	3	2.9 ± 0.3[a]
		Peanut oil	3	1.5 ± 0.1[a]
Schlemitz & Pfannhauser (1996b)	Milk products	Alp-cheese I	3	ND
		Alp-cheese II	3	ND
		Smoked cheese	3	ND
Ohnishi *et al.* (1986)	Fish	Grilled fish	3	< 0.00003–0.00035
		Grilled mackerel	1	0.45
Ohnishi *et al.* (1986)	Meat	Bacon		0.012
		Beef with sauce	1	0.00050
Ohnishi *et al.* (1986)	Chicken meat	Chicken (Yakitori)	1	0.00009
		Chicken white with sauce	1	0.00151
		Chicken (Yakitori), grilled for 3 min	1	0.0038
		Chicken (Yakitori), grilled for 5 min	1	0.019
		Chicken (Yakitori), grilled for 7 minute	1	0.043
Ohnishi *et al.* (1986)	Pork meat	Pork	1	0.00066
		Pork with sauce	1	0.00313

Table 1.5 (continued)

Reference	Category	Food stuff or beverage	No. of samples	1-NP (µg/g)
Schlemitz & Pfannhauser (1996a, b)	Pork meat	Grilled meat (pork)	–	1.0
		Grilled sausages	3	1.4
		Smoked meat (pork)	–	2.2
		Smoked sausages	–	4.2
		Roasted meat (pork)	3	0.3
Schlemitz & Pfannhauser (1996a, b)	Turkey meat	Roasted turkey		ND
Schlemitz & Pfannhauser (1997)	Tea	Assam	3	2.32
		Earl grey	3	7.75
		Ceylon	3	1.54
		Darjeeling	3	4.00
		Mate (roasted)	3	37.89
		Mate (green)	3	0.80
		Formaosa Sencha (green)	3	3.10
		Nettle leaf	3	1.96
		Peppermint	3	3.79
		Fennel (instant)	3	ND
		Fruit (instant)	3	0.55

[a] Fumes from cooking oils in µg/m^3

1-NP, 1-nitropyrene; ND, not detected

1998), and lower levels were detected in grilled food (< 0.03–11.90 ng/g).

1.4.9 Tobacco smoke

The presence of nitroarenes in cigarette-smoke condensate is improbable because the combustion of tobacco during smoking is reductive. 1-Nitropyrene was not found in cigarette smoke using analytical methods with a limit of detection of 1 ng/cigarette (el-Bayoumy *et al.*, 1985) or 10 pg on-column (Williams *et al.*, 1986), or in five commercial brands of cigarette with a limit of detection of 30 pg/cigarette (Scheepers *et al.*, 2001).

1.5 Exposure

1.5.1 Exposure of the general population

(a) Ambient air

After 2-nitrofluoranthene, 1-nitropyrene is the most abundant of 28 mononitro- and dinitro-PAHs identified in airborne PM (Bamford *et al.*, 2003). Its occurrence in the ambient air originates primarily from combustion sources (Atkinson *et al.*, 1991), and in particular from diesel engines (Arey *et al.*, 1986; Zielinska *et al.*, 1986). Photochemical formation in the atmosphere was reported for 2-nitropyrene, but not for 1-nitropyrene (Arey *et al.*, 1987).

An extensive overview of ambient air concentrations was made available (IPCS, 2003), the most recent reports from which are presented below (Table 1.6). Air concentrations of 1-nitropyrene appeared to be associated with sources from urban areas, and more specifically with traffic sources. No 1-nitropyrene was detected in Antarctica or Nepal (Ciccioli *et al.*, 1995). The lowest concentrations detected (< 2 pg/m^3) were observed at 'remote' locations, such as nature reserves in Brazil, the Netherlands, the United Kingdom and the USA (Arey *et al.*, 1988; Ciccioli *et al.*, 1995). Concentrations in suburban and urban areas varied over several orders of magnitude. The highest outdoor concentrations were reported in cities with heavy traffic such as Algiers, Damascus, Milan, Santiago and Tokyo (Tokiwa *et al.*, 1983; Tanabe *et al.*, 1986; Cecinato *et al.*, 1998; Yassaa *et al.*, 2001). Only a few studies reported indoor levels of 1-nitropyrene in homes. Geometric mean indoor concentrations were reported to be 0.67 pg/m^3 in urban residences in Southampton (United Kingdom) and 0.28 pg/m^3 in rural residences in small villages (Scheepers *et al.*, 1999).

(b) Human tissues

Tokiwa *et al.* (1993) retrieved tissues from 137 Japanese nonsmokers (97 men and 40 women) whose cause of death had been registered as lung cancer (squamous cell, small cell and adenocarcinoma), and compared them with 21 specimens from lung cancers with similar histology recovered from Chinese women (aged 28–64 years) who had lived in Wuyuan County, an area with known higher mortality from lung cancer than other regions, and had been farmers and cooks, primarily exposed to soot derived from indoor heating and cooking using coal. The mean 1-nitropyrene content (± standard deviation) per gram of lung tissue of these women (5.9 ± 2.4 pg/g) was much lower than the levels observed in the Japanese subjects (21.3 ± 12.4 pg/g). In contrast, the concentration of benzo[*a*]pyrene was higher in the Chinese women (608.7 ± 447.1 pg/g) than in the Japanese subjects (180 ± 103.7 pg/g).

Toriba *et al.* (2007) developed a liquid chromatography-MS/MS method to determine the urinary metabolites of 1-nitropyrene that was used on urine samples from 17 men and five women living in the city of Kanazawa, Japan (no occupations associated with diesel exhaust emissions or smoking status were reported). Five metabolites of 1-nitropyrene were found (see Section 4.1.1), mostly as glucuronide or sulfate conjugates, including 117, 109, 203, 137 and ≤ 0.54 pmol/mol of creatinine of 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene, 6- and 8-hydroxy-1-nitropyrene and 3-hydroxy-1-nitropyrene, respectively.

Table 1.6 Concentrations of 1-nitropyrene in ambient air

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Industrial						
Morita *et al.* (1982)	Japan	Industrial area	–	1		0.0208
Gibson (1986)	River Rouge, MI, USA	Heavy industrial site	Summer 1982	5	0.59 ± 0.56	0.057
	Dearborn, MI, USA	Heavy industrial site	Summer 1980	8	0.15 ± 0.13	0.029
Atkinson *et al.* (1988)	Yuba City, USA	Biomass burning	Autumn 1986	3	–	0.009
	Concord, USA	-	Winter 1986–87	5	–	0.029
	Mammoth Lakes, USA	Wood smoke	Winter 1987	1	–	0.008
	Oildale, USA	Oil production	Summer 1987	3	–	0.007
Yassaa *et al.* (2001)	Oued Smar, Algeria	Landfill	Winter 1999	1	–	0.080
			Summer 1998	1	–	< 0.01
Urban–suburban						
Gibson (1982)	Detroit, MI, USA	Suburban	Spring and summer 1981	2	0.27 0.18	0.016 0.030
	Warren, MI, USA	Suburban	Spring and summer 1981	2	0.56 0.42	–
Tokiwa *et al.* (1983)	Santiago, Chile	Downtown	Winter 1981	4	0.06–0.15	0.028–0.11
Siak *et al.* (1985)	Michigan, USA	–	Summer	4	0.037–0.11	0.0024–0.012
Garner *et al.* (1986)	Bayreuth, Germany	Suburban	November 1983	2	–	1.5; 1.7
Tanabe *et al.* (1986)	Tokyo, Japan	Downtown	February	2	1.6; 0.579	0.041–0.13
			April	2	0.30–0.83	0.032–0.062
			August	2	0.19–0.90	0.015–0.038
			October	2	0.61–0.75	0.051–0.080
Ramdahl *et al.* (1986)	Claremont, CA, USA	Suburban	Summer 1985	1	0.36	–
	St Louis, MO, USA	Urban	Sampled over 1 year	1	0.16	–
	Washington DC, USA	Urban	Sampled over 1 year	1	0.20	-
Gibson (1986)	Warren, MI, USA	Suburban	Winter 1982	7	0.36 ± 0.15	0.015
			Summer 1984	5	0.35 ± 0.12	0.022
	Detroit, MI, USA	Urban	Summer, 1981	15	0.22 ± 0.20	0.030
Arey *et al.* (1987)	Torrance, CA, USA	Suburban	Winter daytime	1	–	0.04
			Winter night-time	1	–	0.03

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Atkinson *et al.* (1988)	Glendora, USA	–	Summer 1986	6	–	0.020
	Reseda, USA	–	Summer 1987	2	–	0.008
Zielinska *et al.* (1989)	Claremont, USA	Suburban	Autumn	1	–	0.016
Bayona *et al.* (1994)	Barcelona, Spain	–	1989–90	1	–	0.026
Scheepers *et al.* (1994a)	Nijmegen, Netherlands	Busy street crossing	February, 1992	1	–	0.036
Legzdins *et al.* (1995)	Hamilton, Canada	–	Spring/summer 1990–91	1	–	0.012
Ciccioli *et al.* (1995)	Madrid, Spain	Suburban	Autumn 1991–93	1	–	0.010
	Montelibretti, Italy	Suburban	1991–93	1	–	0.012
	Milan, Italy	–	Winter 1991–93	1	–	0.220
	Rome, Italy	–	Winter 1991–93	1	–	0.070
	Sao Paulo, Brazil	–	Winter 1991–93	1	–	0.016
Dimashki *et al.* (1996)	Damascus, Syrian Arab Republic	–	Winter 1994	1	–	0.120
Murahashi & Hayakawa (1997)	Kananazawa, Japan	Suburban	Summer 1994	1	–	[0.010]
		Downtown	Summer 1994	1	–	[0.032]
Cecinato *et al.* (1998)	Milan, Italy	Viale Marche	Winter 1991	1	–	0.14
	Rome, Italy	Brera Tower	Winter 1993	1	–	0.59
		via Urbana	Summer 1991–1993	1	–	0.08
Scheepers *et al.* (1999)	Southampton, United Kingdom	Daytime	Weekday in May 1998	13	–	[0.0086 ± 0.0021[a]]
		Night-time		9	–	[0.0011 ± 0.0024[a]]
		Urban residence, indoor 1st floor	Night-time	4	–	[0.00067 ± 0.0053[a]] (< 0.001–0.0038)
		Urban	May 1998	3	–	0.0012–0.0035
	Kananazawa, Japan	Urban	Summer 1994	1	–	0.032

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Marino *et al.* (2000)	Athens, Greece	–	1996	1	–	0.040
Yassaa *et al.* (2001)	Algiers, Algeria	Urban	Winter 1999	1		0.14
Bamford *et al.* (2003)	Washington, DC, USA (SRM1649a)	Urban	1970s	3	0.0715 ± 0.0051	–
	St. Louis, MO, USA (SRM1648)	Urban	1978	3	0.155 ± 0.029	–
	Baltimore, MD, USA	Urban	1998–99	3	0.196 ± 0.003	–
Ari *et al.* (2010)	Payas, Turkey	Urban industrial				
		PM-10 fraction	May 2008	7	0.212 (0.144–0.323)[b]	0.0059 (0.0037–0.0096)[b]
		PM-2.5 fraction	May 2008	7	0.191 (0.103–0.246)[b]	0.0024 (0.0014–0.0137)[b]
	Iskenderun, Turkey	Urban background; PM-10 fraction	May 2008	8	0.273 (0.108–0.621)[b]	0.0041 (0.0007–0.0123)[b]
	Eskişehir, Turkey	Urban road side; PM-10 fraction	May–June 2008	5	0.930 (0.168–1,270) [b]	0.0192 (0.0066–0.0229)[b]
Rural						
Nielsen (1983)	Copenhagen, Denmark	Rural	February–April 1982			0.02 (< 0.001–0.04)
Gibson (1986)	Delaware, USA	Rural	Summer 1982		0.54 ± 0.24	0.013
	Bermuda	Remote	Summer 1982	1	0.52 ± 0.29	0.0096
			Winter 1983	1	0.72 ± 0.43	0.0103
Ramdahl *et al.* (1986)	Aurskog, Norway	Rural residential	Winter, 1984	1	0.15	–
Atkinson *et al.* (1988)	Pt. Arguello, USA	Remote	Summer, 1987	2	–	0.0005
	San Nicolas Island, USA	Remote	Summer 1987	1	–	0.0003
Saitoh *et al.* (1990)	Morioka, Japan	Rural	November–April		–	0.038–0.245
Ciccioli *et al.* (1995)	Castelporziano, Italy	Forest area	Winter 1992	1	–	0.002
	Alta Floresta, Brazil	Not specified	Winter/spring 1993	1	–	0.002
Scheepers *et al.* (1994a)	Hoenderloo, Netherlands	Nature reserve area	July 1992	1	0.034	0.0017

Table 1.6 (continued)

Reference	Location	Description	Season and/or year	No. of samples	1-NP (μg/g) (AM ± SD)	1-NP (ng/m³)
Scheepers *et al.* (1999)	Southampton, United Kingdom	Rural	Night-time, weekend May 1998	3	–	0.0005 0.0006 0.0008
	New Mylton, United Kingdom	Rural residence, indoor	Night-time	6	–	[0.0028 ± 0.0088][a] (< 0.0001–0.0086)
Cecinato *et al.* (2000)	Svalbard Island, Norway	Remote	1998–99	1	–	0.016
Albinet *et al.* (2006)	Sollieres, Maurienne Valley, France, 1373 m altitude	Rural	Winter 2002–03	13	–	0.0106 (0.0027–0.0289)
Ari *et al.* (2010)	Eskişehir, Turkey	PM-10 fraction	May–June 2008	4	0.30 (0.18–0.50)[b]	0.0048 (0.0028–0.0062)[b]
		PM-2.5 fraction	May–June 2008	4	0.21 (0.11–0.49)[b]	0.0029 (0.0012–0.0060)[b]

[a] Geometric mean and geometric standard deviation calculated by the Working Group; for results reported as 'not detected', half of the limit of quantification was used; the classification by the Working Group was based on Google Maps.

[b] Median and range

1-NP, 1-nitropyrene; PM, particulate matter; SRM, standard reference material

3-Hydroxy-*N*-acetyl-1-aminopyrene, 1-aminopyrene and *N*-acetyl-1-aminopyrene were not detected with this method.

1.5.2 Occupational exposure to engine exhaust

(a) Personal air sampling

Workers who operate or maintain diesel-powered engines or vehicles are liable to be exposed to their exhaust. Most measurements were obtained by high-volume sampling because of the low levels observed in air, specifically at outdoor locations. Table 1.7 summarizes air concentrations of 1-nitropyrene determined at fixed outdoor sampling locations, where different types of diesel engine were used. Workers often try to avoid remaining downwind from sources of exhausts, which, together with dilution by ambient air, maintains their exposure at relatively low levels, compared with those found indoors, and within the range of those observed in the general ambient air of most urban locations (usually < 1 ng/m^3; see Table 1.6).

At indoor workplaces, exposures tend to be higher (occasionally > 1 ng/m^3; (Table 1.7), with the exception of engine repair shops, where exhaust is removed by connecting the tailpipe to an effective local exhaust ventilation system. When the capacity of this system is insufficient, levels of 1-nitropyrene may reach > 5 ng/m^3 (Scheepers *et al.*, 1994a). Overall, train repair shops and mining operations represented indoor workplaces with the highest exposures (average exposure levels, > 1 ng/m^3; see Fig. 1.1).

A few studies that attempted to determine exposure to 1-nitropyrene by taking personal air samples from the breathing zone of workers (Table 1.8) were only feasible when the analysis used was sensitive, because the airflow that can be used in compact air suction pumps is limited. The exposure of mechanics at a bus garage in Southampton (United Kingdom) was relatively low, but sampling periods were long and included the time spent commuting from home to work and vice versa [which may have underestimated the contribution of exposure in the workplace, where levels were probably higher than during commuting]. Mechanics had higher exposures than office clerks, and, on average, more than 75% of the exposure resulted from working in the repair shop. The mean contribution of local outdoor concentrations of 1-nitropyrene to the average exposure of workers was 16.1% (Scheepers *et al.*, 1999).

The highest exposures to 1-nitropyrene were observed in oil-shale mining (Estonia) and black coal mining (Czech Republic). Underground oil-shale miners were exposed to levels of 1-nitropyrene one to two orders of magnitude higher than those of workers employed in surface operations, including indoors. The personal air samples of workers exposed to exhausts from loaders that were continuously moving back and forth in narrow confined spaces contained concentrations of up to 40 ng/m^3 of 1-nitropyrene. In coal mines, the workers were exposed to exhausts from trains used for the transportation of coal and personnel that contained concentrations of 1-nitropyrene ranging up to 2.5 ng/m^3, which on average were much lower than those detected in oil-shale mining; no difference was found in average exposures to 1-nitropyrene between underground miners and workers in surface operations (Scheepers *et al.*, 1993, 1994a).

(b) Biomonitoring

Levels of 2–200 ng of 1-aminopyrene, a urinary metabolite of 1-nitropyrene, were observed in 24-hour urine samples of nine smoking and nine nonsmoking salt miners exposed to diesel exhaust in Germany (Seidel *et al.*, 2002). Seidel *et al.* (2002) and Scheepers *et al.* (1995b) developed an immunoassay to detect the urinary excretion of 1-aminopyrene, which was used in a study of three nonsmoking mechanics and two nonsmoking office clerks in a repair workshop for trains (Scheepers *et al.*, 1994b), from whom 24-hour urine samples were collected over 3 days. Combined urine aliquots

Table 1.7 Concentrations of 1-nitropyrene in extracts of total suspended particulate matter associated with the use of diesel engines collected at workplaces

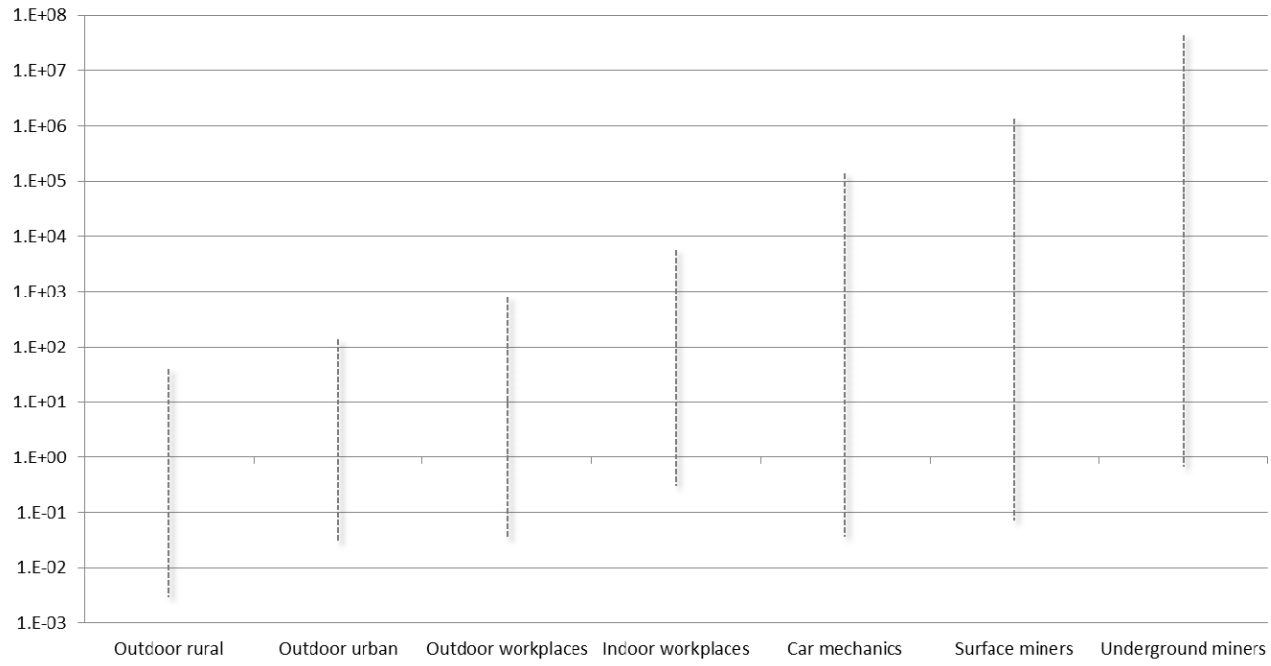
Reference	Job title	Source	Working conditions	Location and conditions	Year	No. of samples	1-NP (ng/m³)[a] (range)
Outdoor							
Scheepers *et al.* (1994a)	Professional driver	Armoured cars	Training in field operation of armed vehicle driving	Netherlands; wind speed 4–6 m/s	1992	2	0.012; 0.015
	Ship's crew	Ship's engine	River vessel	Netherlands and Belgium; wind speed 8–9 m/s	1992	3	0.034 ± 0.0019
		Power supply			1992	1	0.79
	Gardener	Passing traffic	City park	Netherlands	1992	1	0.036
		Lawn mowers	Grass verge maintenance	Netherlands	1992	1	0.0066
	Platform personnel	Lift platforms, power supplies, trucks, air craft push back tractors	Platform of Amsterdam international airport	Schiphol, Netherlands	1992	3	0.034 ± 0.0014 (0.037–0.045)
Indoor							
Scheepers *et al.* (1999)	Mechanics	Buses	Garage	United Kingdom	1998	14	0.054 ± 2.4 (0.010–0.227)
			Fitting shop			13	0.053 ± 2.0 (0.015–0.120)
Scheepers *et al.* (1994a)	Mechanics	Train engines	Repair workshop	Netherlands	1992	4	0.31 ± 1.2 (0.26–0.39)
					1994	7	1.8 ± 2.4[a] (0.5–5.6)
	Logistic personnel	Trucks	Flower auction building	Netherlands	1992	1	0.08
	Driver	Forklift truck	Concrete manufacturing			2	0.61; 0.71
			Aluminium rolling			4	1.1 ± 1.1 (0.87–1.2)
			Galvanization workshop			4	0.09 ± 1.7 (0.044–0.15)
			Chemical plant			4	0.22 ± 0.21 (0.11–0.56)
Seidel *et al.* (2002)	Miner	Not specified	Underground salt mine	Germany		3	0.70; 1.3; 1.00

Table 1.7 (continued)

Reference	Job title	Source	Working conditions	Location and conditions	Year	No. of samples	1-NP (ng/m³)[a] (range)
Scheepers *et al.* (2002, 2003)		Train	Coal mining (surface operations)	Czech Republic	1999	8	0.0028 ± 0.0037 (0.007–0.185)
			Coal mining (underground)			8	0.078 ± 0.0033 (0.028–0.697)
		Loader	Oil-shale mine (surface operations)	Estonia		6	0.070 ± 0.0021 (0.025–0.193)
			Oil-shale mine (underground)			5	0.216 ± 0.002 (0.111–0.506)

[a] Geometric mean and geometric standard deviation summarizing observations covering three workweeks, calculated by the Working Group; for results reported as 'not detected', half of the limit of quantification was used.
1-NP, 1-nitropyrene

Fig. 1.1 Range of air concentrations of 1-nitropyrene (in ng/m³)



The values for car mechanics, surface miners and underground miners were measured in the breathing zone by personal air sampling. Other values were the results of air sampling at fixed locations (stationary high-volume sampling). The results presented in this figure are also summarized in Tables 1.4–1.7.
Compiled by the Working Group.

of mechanics showed an almost twofold increase in the level of excreted 1-aminopyrene (expressed as nanomolar equivalents; $P < 0.05$) after 48 and 72 hours, and also after adjustment for creatinine ($P < 0.05$), compared with the levels excreted by office clerks.

Only one study described the determination of 1-aminopyrene in blood samples (Zwirner-Baier & Neumann, 1999) following the hydrolysis of haemoglobin adducts. Before hydrolysis, the erythrocytes were washed with water and 0.9% saline, and proteins were then precipitated and rinsed with aliquots of ethanol:water (80:20), ethanol and ethanol:ether (25:75) after lysis of the cells. [It is not clear to what extent free metabolites (from plasma and erythrocytes) were removed by this pretreatment, or may have contributed to the 1-aminopyrene content.] The blood samples were from 63 male nonsmokers, divided into three subgroups: 29 bus garage workers, 20 hospital workers and 14 men living in rural areas around Southampton, United Kingdom. 1-Aminopyrene was detected in 86–100% of the subjects, with levels ranging from < 0.03 to 0.68 pmol/g of haemoglobin. 1-Aminopyrene was the most abundant nitroarene metabolites (other cleavage products detected were 2-aminofluorene, 3-aminofluoranthene, 9-aminophenanthrene and 6-aminochrysene). Interestingly, the highest median levels of 1-aminopyrene were observed in hospital workers (0.16 pmol/g), followed by bus garage workers (0.13 pmol/g) and inhabitants of a rural environment (0.10 pmol/g).

1.6 Regulations and guidelines

No known air quality guidelines or reference values have been established by national or international authorities for 1-nitropyrene.

Table 1.8 Concentrations of 1-nitropyrene determined by personal air sampling in the breathing zone of workers

Reference	Source	Working conditions	Location and conditions	Year	No. of workers	Concentration of 1-NP (pg/m³)	
						AM ± SD	GM ± GSD (range)
Car mechanics							
Scheepers *et al.* (1999)	Buses	Work shift in bus garage including the time commuting from home to work and vice versa	Southampton, United Kingdom	1998	5	52 ± 21[a]	48 ± 1.6[a]
						19 ± 16[a]	4.0 ± 8.1[a]
						8.0 ± 9.2[a]	2.2 ± 6.0[a]
						36 ± 32[a]	8.5 ± 10.2[a]
						12.9 ± 6.9[a]	7.8 ± 4.0[a]
Miners							
Seidel *et al.* (2002)	Not specified	Salt mine	Germany		4	[1171 ± 867][b]	(972 ± 740][b]
Scheepers *et al.* (2002, 2003)	Loaders	Oil shale mine, surface operations	Estonia				
			Pilot study (1st shift)	1999	9	–	8 ± 2.3 (3–50)
			Pilot study (2nd shift)	1999	9	–	45 ± 3.8 (12–686)
			Main study (3 shifts)	2000	42	–	85 ± 2.7 (13–1332)
		Oil shale miners, underground	Estonia				
			Pilot study (1st shift)	1999	10	–	2483 ± 3.4 (602–42 190)
			Pilot study (2nd shift)	1999	10	–	984 ± 2.7 (134–3455)
			Main study (3 shifts)	2000	50	–	637 ± 3.3 (29–5031)
	Trains	Coal mine, surface operations	Czech Republic				
			Pilot study (1st shift)	1999	10	–	110 ± 2.8 (19–666)
			Pilot study (2nd shift)	1999	9	–	71 ± 7.6 (7–2167)
		Coal mine, underground	Czech Republic				
			Pilot study (1st shift)	1999	9	–	197 ± 1.5 (123–437)
			Pilot study (2nd shift)	1999	9	–	209 ± 3.6 (28–2495)

[a] Mean over one workweek (4–5 days) calculated by the Working Group; for results reported as not detected, half of the limit of quantification was used.
[b] Calculated by the Working Group; combination of stationary and personal air samples.
AM, arithmetic mean; GM, geometric mean; GSD geometric standard deviation; 1-NP, 1-nitropyrene; SD, standard deviation

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Subcutaneous administration

A group of 20 male BALB/C mice, aged 6 weeks, received subcutaneous injections of 0 (control) or 0.1 mg of 1-nitropyrene (purity, > 99.9%) dissolved in dimethyl sulfoxide (DMSO) once a week for 20 weeks (total dose, 2 mg), and were observed for up to 60 weeks after the initial treatment or until moribund. No subcutaneous tumours developed at the injection site in any animals. Lung tumours developed in 6 out of 20 (30%) 1-nitropyrene-treated and 7 out of 20 (35%) control mice (Tokiwa *et al.*, 1984). [The Working Group noted the small number of animals used in this study.]

3.1.2 Intraperitoneal administration

Three groups of 12–16 male and 12–16 female A/J mice, aged 6–8 weeks, received a total of 17 intraperitoneal injections (three per week) of 0 (control), 0.71, 2.14 of 6.14 mmol/kg body weight (bw) of 1-nitropyrene (purity, > 99%) in 0.1 mL of trioctanoin over a period of 6 weeks (total doses, 0, 175, 525 and 1575 mg/kg bw, respectively), and were killed 18 weeks after termination of the treatment (total, 24 weeks). The number of high-dose males with lung tumours (14 out of 16) was significantly greater [$P < 0.001$] than that of male controls (3 out of 16). The mean number of lung tumours per mouse was also significantly increased in the low- (0.6 ± 0.5) and high- (1.6 ± 1.1) dose males compared with male controls (0.3 ± 0.6; $P < 0.001$). The mean number of lung tumours per mouse was also significantly increased in high-dose females (0.8 ± 0.8) compared with female controls (0.3 ± 0.6; $P < 0.05$) (el-Bayoumy *et al.*, 1984).

Male and female newborn CD-1 mice [initial numbers unspecified] received three intraperitoneal injections of 1-nitropyrene (purity, > 99%) on day 1 (100 or 400 nmol), day 8 (200 or 800 nmol) and day 15 (400 or 1600 nmol) in DMSO (total doses, 700 or 2800 nmol [173 or 692 µg] in 20 or 40 µL of DMSO/mouse) after birth or DMSO alone. A positive control group received a total dose of 560 nmol [140 µg] of benzo[*a*]pyrene (purity, > 99%) in DMSO. All surviving mice were killed after 1 year. Hepatocellular adenomas or carcinomas (combined) developed in 5 out of 34 (15%) low-dose (two adenomas and three carcinomas) and 8 out of 29 (27%) high-dose 1-nitropyrene-treated males; the latter incidence was significantly greater than that in DMSO controls (8 out of 29 versus 2 out of 28; $P < 0.05$). No liver tumours developed in 1-nitropyrene-treated females. The incidence of lung tumours did not differ significantly in 1-nitropyrene-treated males (6 out of 34 low-dose and 1 out of 29 high-dose) or females (3 out of 50 low-dose and 2 out of 26 high-dose) compared with controls (1 out of 28 males and 0 out of 31 females), nor did that of malignant lymphomas (males: 4 out of 28 control, 0 out of 34 low-dose and 7 out of 29 high-dose; females: 3 out of 31 control, 6 out of 50 low-dose and 4 out of 26 high-dose) (Wislocki *et al.*, 1986).

Groups of newborn male and female Swiss-Webster BLU:Ha [ICR] mice [initial numbers unspecified] received three intraperitoneal injections of 1-nitropyrene (purity, > 99.9%) in DMSO or DMSO alone on day 1 (1/7th of the dose), day 8 (2/7th of the dose) and day 15 (4/7th of the dose) after birth (total doses: 21 µg (0.08 µmol) in 35 µL of DMSO/mouse or 105 µg (0.43 µmol) in 35 µL of DMSO/mouse). The mice were then maintained untreated for 24 weeks and killed at

Table 3.1 Studies of the carcinogenicity of 1-nitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
BALB/c (M) 60 wks Tokiwa *et al.* (1984)	Subcutaneous injection 0 (control) or 0.1 mg/mouse in DMSO once/wk for 20 wks (total dose, 2.0 mg/mouse) then observed up to 60 wks Groups of 20	Injection site (subcutaneous tumour): 0/20, 0/20 Lung (adenoma or carcinoma combined): 7/20 (35%), 6/20 (30%)	NS	Purity, > 99.9% by HPLC
A/J derived (M, F) 24 wks El Bayoumy *et al.* (1984)	Intraperitoneal injection 0 (control), 0.71, 2.14 or 6.44 mmol/kg bw in 0.1 mL trioctanoin, 3 ×/wk for 6 wks (total of 17 injections; total doses: 175, 525 or 1575 mg/kg bw) then held untreated for an additional 18 wks Groups of 12–16	Lung (all tumours): M–3/16 (19%), 4/15 (27%), 6/15 (40%), 14/16 (86%) F–4/16 (25%), 3/14 (21%), 5/14 (36%), 8/12 (67%) Multiplicity (lung tumours): M–0.3 ± 0.6, 0.6 ± 0.5, 0.6 ± 0.8, 1.6 ± 1.1 F–0.3 ± 0.6, 0.2 ± 0.4, 0.4 ± 0.7, 0.8 ± 0.8	M–[high dose, $P < 0.001$] F–[NS] M–[low dose, $P < 0.05$; high dose, $P < 0.001$] F–[high dose, $P < 0.05$]	Purity, > 99% by TLC; small number of animals, short durations of treatment and observation
CD-1 (F, M) 52 wks Wislocki *et al.* (1986)	Intraperitoneal injection 0 (control), 700 or 2800 nmol 1-NP or 560 nmol B[*a*]P in 10, 20 or 40 µL DMSO (total doses) on d 1, 8 and 15 after birth Groups of 28–50 newborn	Liver (adenoma): M–7/73 (10%), 2/34 (6%), 3/29 (10%)*, 11/37 (30% ; B[*a*]P) F–0/65, 0/50, 0/26, 0/27 (B[*a*]P) Lung (carcinoma): M–0/73, 3/34 (9%), 5/29 (17%)*, 7/37* (19%; B[*a*]P) F–0/65, 0/50, 0/26, 0/27 (B[*a*]P) Lung (adenoma): M–3/73 (4%), 6/34 (18%), 1/29 (3%), 13/37 (35%; B[*a*]P) F–1/65 (1%), 3/50 (6%), 2/26 (8%), 13/27 (48%; B[*a*]P) Lung (carcinoma): M–3/73 (4%), 0/34, 0/29, 0/37 (B[*a*]P) F–1/65 (1%), 0/50, 0/26, 0/27 (B[*a*]P)	*$P < 0.05$	Purity, > 99% by HPLC

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Swiss Webster (M, F) 26 wks Busby *et al.* (1989)	Intraperitoneal injection 0 (control), 21 µg (0.08 µmol) or 105 µg (0.43 µmol) in 35 µL DMSO/mouse (total doses); 1/7 dose at d 1 after birth, 2/7 dose on d 8, 4/7 dose on d 15 Groups of newborn (NR)	Lung (adenoma or adenocarcinoma combined): M–13/91 (14%), 2/29 (7%), 1/23 (4%) F–7/101 (7%), 3/23 (13%), 0/30	NS	Purity > 99.9%
B6C3F$_1$ (M, F) 72 wks Mori *et al.* (1992)	Intraperitoneal injection 0 (control), 100 nmol within 24 h of birth, 200 nmol on d 8, 400 nmol d 15 (total dose, 700 nmol/mouse); untreated and control also available Groups of (NR) newborn	Liver (hepatocellular adenoma): M–1/39 (3%; untreated), 4/28 (14%), 4/21 (19%) F–0/38 (untreated), 0/27, 0/20	NS	Purity > 99.9% by HPLC
CD-1 (F) 28 wks el-Bayoumy *et al.* (1982)	Initiation–promotion: skin application 0 (control) or 0.1 mg in 0.1 mL acetone onto shaved back skin, once/2 d for 20 d (10 doses; total dose, 1 mg); 10 d later 2.5 µg TPA in 0.1 mL acetone 3 ×/wk for 25 wks Groups of 20	Skin (mainly squamous-cell papillomas): [3/19] (16%), 1/20 (5%) Multiplicity (skin tumours): 0.2, 0.1	NS	Purity > 99%
SENCAR (M, F) 31 wks Nesnow *et al.* (1984)	Initiation–promotion: skin application/ intraperitoneal injection 0 (control), 0.03, 0.10, 0.30 or 1.00 mg (once) or 3.00 mg (1.50 mg twice) in 0.2 mL acetone (skin) or 0.2 mL corn oil (injection); 1 wk later 2 µg TPA, twice/wk (skin) for 30 wks Groups of 37–40 M, 37–40 F	Skin (papillomas per effective mouse): M–0.06 (31), 0.11 (38), 0.18 (40), 0.08 (39), 0.11 (38), 0.10 (39) F–0 (39), 0.06 (36), 0.28 (40), 0 (38), 0.10 (39), 0.21 (38)	NS NS	Purity > 99.9% (HPLC)

B[*a*]P, benzo[*a*]pyrene; bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; HPLC, high-performance liquid chromatography; M, male; 1-NP, 1-nitropyrene; NR, not reported; NS, not significant; TLC, thin-layer chromatography; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week

26 weeks of age. The incidence of lung adenomas or adenocarcinomas (combined) was 13 out of 91 (14%), 2 out of 29 (7%) and 1 out of 23 (4%) males, and 7 out of 101 (7%), 3 out of 23 (13%, all adenocarcinomas) and 0 out of 30 females in the control, 21-µg and 105-µg groups, respectively, showing no difference between controls and treated animal (Busby *et al.*, 1989).

Male and female newborn B6C3F$_1$ mice [initial numbers unspecified] received intraperitoneal injections of 1-nitropyrene dissolved in DMSO or DMSO alone on day 1 (100 nmol), day 8 (200 nmol) and day 15 (400 nmol) after birth. An untreated control group was also available. Surviving animals were killed after 72 weeks. The incidence of hepatocellular adenomas in treated males (4 out of 21, 19%) and females (0 out of 20) was not statistically different from that in DMSO-treated controls (4 out of 28, 14% and 0 out of 27, respectively) (Mori *et al.*, 1992).

3.1.3 Initiation–promotion

Group of 20 female CD-1 Charles River mice, aged 50–55 days, received applications of 0 (control) or 0.1 mg of 1-nitropyrene (purity > 99%) in 0.1 mL of acetone onto the shaved back skin by pipette every other day for 20 days (total dose, 1 mg). Ten days after the completion of initiation, the animals received applications of 2.5 of µg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL of acetone three times a week for 25 weeks. The mice were killed 28 weeks after the first treatment with 1-nitropyrene. Neither the incidence of skin tumours (mainly squamous cell papillomas; 16% [3 out of 19] versus 5% [1 out of 20]) nor the multiplicity of skin tumours per mouse (0.2 versus 0.1) in 1-nitropyrene-treated mice differed from those in controls (el-Bayoumy *et al.*, 1982).

Groups of 37–40 male and 37–40 female SENCAR mice, aged 7 weeks, initially received treated a single dermal application of 0 (control), 0.03, 0.10, 0.30 or 1.00 mg/mouse of 1-nitropyrene (> 99.9% pure) dissolved in 0.2 mL of acetone onto the back. The highest-dose group received 3.0 mg of 1-nitropyrene as two applications of 1.5 mg each. One week later, the mice received applications of 2 µg of TPA in 0.2 mL of acetone twice a week for 30 weeks. For comparison of tumour yield, a positive-control group was pretreated with 0.051 mg/mouse of benzo[*a*]pyrene. The number of papillomas/mouse for each dose group was 0.06, 0.11, 0.18, 0.08, 0.11 and 0.10 in males and 0, 0.06, 0.28, 0, 0.10 and 0.21 in females, respectively. Thus, dermal application of 1-nitropyrene did not act as a skin tumour initiator (Nesnow *et al.*, 1984).

3.2 Rat

See Table 3.2

3.2.1 Oral administration

Groups of 22–36 male and 24–33 female newborn Sprague-Dawley rats were administered 0 (control), 100 or 250 µmol/kg bw of 1-nitropyrene (320 µmol/rat in 16.2 µmol of trioctanoin or 800 µmol/rat in 40.5 µmol of trioctanoin) by gavage within 24 hours of birth and then once a week for 16 weeks. Animals were then maintained without treatment and were killed at week 96. The incidence of mammary gland adenocarcinomas was increased in low-dose (14 out of 33, 42%) and high-dose (15 out of 24, 62%) females compared with controls (1 out of 31, 3%; $P < 0.0001$). The incidence of lung adenomas or adenocarcinomas (combined) in males was increased in the high-dose group only (0 out of 22 versus 4 out of 36, 11%; $P < 0.05$) (el-Bayoumy *et al.*, 1988).

Groups of 35 female weanling Sprague-Dawley rats were administered 0 (control) or 10 µmol/kg bw of 1-nitropyrene in DMSO by intragastric intubation three times a week for 4 weeks (total dose, 16 µmol/rat in 1.7 µmol/mL of DMSO) and were then observed with no further treatment until they were killed at 78 weeks.

Table 3.2 Studies of the carcinogenicity of 1-nitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
SD (F, M) 96 wks el-Bayoumy *et al.* (1988)	Oral administration 0 (control), 100 (320 µmol/rat in 16.2 µmol/mL trioctanoin) or 250 µmol/kg bw (800 µmol/rat in 40.5 µmol/mL trioctanoin) by gavage within 24 h of birth, once/wk for 16 wks, then observed up to 96 wks Groups of 22–36 M, 22–36 F	Mammary gland (adenoma): M–1/22 (4%), 2/25 (8%), 1/36 (3%) F–14/31 (45%), 10/33 (30%), 7/24 (29%) Mammary gland (adenocarcinoma): M–0/22, 2/25 (8%), 2/36 (6%) F–1/31 (3%), 14/33 (42%), 15/24 (62%)* Lung (adenoma or adenocarcinoma combined): M–0/22, 1/25 (4%), 4/36 (11%) F–0/31, 0/33, 3/24 (12%)** Pancreas (islet cell adenoma): M–0/22, 4/25 (16%), 3/36 (8%) F–0/31, 1/33 (3%), 1/24 (4%)	*$P < 0.01$ (high, low) **$P < 0.05$ otherwise, NS	Purity, > 99.9%; other mammary tumours included fibromas, sarcomas and myxomas
CD (F) 78 wks King (1988), Imaida *et al.* (1991a)	Intragastric administration 0 (control) or 10 µmol/kg bw, 3 ×/wk for 4 wks (total dose, 16 µmol/rat) in DMSO then observed to 78 wks Groups of 36 weanling	Mammary gland (all tumours): 12/35 (34%), 16/35 (46%) Mammary gland (fibroadenoma): 9/35 (26%), 9/35 (26%) Mammary gland (adenocarcinoma): 5/35 (9%), 5/35 (9%)	NS	Purity, > 99.9%
SD (F) 49 wks el-Bayoumy *et al.* (1995)	Intragastric administration 0 (control) or 50 µmol in 0.5 mL trioctanoin, once/wk for 8 wks (total dose, 400 µmol/rat), then observed for 41 wks Groups of 30	Mammary gland (fibroadenoma): 0/30, 10/30 (33%)* Mammary gland (desmoplastic adenoma [adenoma criss-crossed by thick bands of fibrous connective tissue]): 8/30 (27%), 15/30 (50%) Mammary gland (adenoma): 2/30 (7%), 7/30 (23%) Mammary gland (adenocarcinoma): 1/30 (33%), 2/30 (67%) Multiplicity (mammary tumours): 0, 15** (fibroadenoma); 14, 29 (desmoplastic adenoma); 2, 10 (adenoma); 1, 3 (adenocarcinoma)	*$P < 0.01$ **$P < 0.05$	Purity, > 99.9%; study limited by use of a single dose and short duration of exposure

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) Duration (NR) Ohgaki *et al.* (1982)	Subcutaneous injection 0 (control) or 20 mg/rat in 0.2 mL DMSO, twice/wk for 10 wks (total dose, 40 mg) Groups of 20	Injection site (subcutaneous tumour[a]): 0/20, 8/17 (47%)	P < 0.003	Purity, > 99%; later, Ohgaki *et al.* (1985) reported that the 1-NP used contained impurities: 1,3-dinitropyrene (DNP) (0.2%); 1,6-DNP (0.3%); 1,8-DNP (0.3%)
CD (SD-derived) (F, M) 62 wks Hirose *et al.* (1984)	Subcutaneous injection 0 (control), 50 or 100 µmol/kg bw in DMSO into the suprascapular area, starting within 24 h of birth, once/week for 8 wks and observed until aged 62 wks Groups of 28–35 newborn	Tumour-bearing animals (all tumours[b]): M–6/28 (21%), 10/29 (34%), 15/31 (48%)* F–8/31 (26%), 19/31 (61%)**, 24/32 (75%)*** Injection site (subcutaneous fibrous histiocytomas): M–0/28, 2/29 (7%), 10/31 (32%)* F–0/31, 3/31 (10%), 9/32 (28%)** Mammary gland (all tumours): F–2/31 (6%), 7/31 (23%), 15/32 (47%)* Mammary gland (fibroadenoma): F–1/31 (3%), 5/31 (16%), 7/32 (22%)* Mammary gland (adenocarcinoma): F–0/31, 3/31 (10%), 10/32 (31%)*	*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *$P < 0.001$; **$P < 0.01$ *$P < 0.001$ (high) *$P < 0.05$ *$P < 0.01$	Purity, 99.9%; other mammary tumours included adenomas and carcinosarcomas
F344/DuCrj (M) Up to 650 d Ohgaki *et al.* (1985)	Subcutaneous injection 0 (control), 0.2 or 2 mg/rat in 0.2 mL DMSO, twice/wk for 10 wks (total dose, 4 and 40 mg), then observed untreated up to 650 days Groups of 10 or 20	Injection site (subcutaneous tumour[a]): 0/20, 0/10, 0/10	NS	Purity checked by HPLC; 1,3-, 1,6-, 1,8-DNP, were minimized to 0.05% each; study limited by the small numbr of treated animals and use of one dose only; authors concluded that tumour incidence in the previous study (Ohgaki *et al.* (1982) was due to contaminants (1,8- and 1,6-DNP) and that 1-NP was not a skin carcinogen following subcutaneous injection.

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 90 wks King (1988), Imaida *et al.* (1991b)	Subcutaneous injection 0 (control) or 100 µmol/kg bw in DMSO (70 µmol/mL) once/wk for 4 wk (total dose, 74.3 µmol), then observed for up to 90 wks Groups of 30	All tumours [b]: 27/30 (90%), 23/29 (79%) Mammary gland (all tumours): 11/30 (37%), 17/29 (57%) Mammary gland (fibroadenoma): 8/30 (27%), 15/29 (48%)* Mammary gland (adenocarcinoma): 4/30 (13%), 6/29 (21%)	*$P < 0.05$	Purity (NR; purchased from the Midwest Research Institute, MO)
CD (F) 67 or 86 wks King (1988), Imaida *et al.* (1995)	Subcutaneous injection 0 (control) or 2.5 (within 24 h of birth), 5 (2 injections) and 10 µmol/kg bw in DMSO into suprascapular region, once/wk for 8 wks (total dose, 6.3 µmol [18 mg]), and observed untreated for up to 78 wk Groups of 37–49 newborn	Mammary gland (all tumours): 8/40 (20%), 16/49 (33%) Mammary gland (adenocarcinoma): 1/40 (2%), 10/49 (20%) Mammary gland (fibroadenoma): 6/40 (15%), 9/49 (18%)	$P < 0.05$ $P < 0.005$ NS	Purity, > 99.9%; no tumours at the injection site; other mammary tumours included adenomas and carcinosarcomas
CD or F344 (F) 67 or 86 wks King (1988), Imaida *et al.* (1995)	Subcutaneous injection 0 (control) or 100 µmol/kg bw in DMSO into suprascapular region, starting within 24 h of birth once/wk for 8 wks (total dose, 800 µmol/kg bw) then observed untreated for up to 78 wks Groups of 48–49 newborn CD; 55 newborn F344	**CD rats** Mammary gland (all tumours): 17/47 (36%), 26/48 (54%) Mammary gland (adenocarcinoma): 3/47 (6%), 10/48 (21%) Mammary gland (fibroadenoma): 16/47 (34%), 19/48 (40%) **F344 rats** Mammary gland (all tumours): 1/55 (2%), 5/55 (9%) Mammary gland (adenocarcinoma): 0/55, 0/55 Mammary gland (fibroadenoma): 1/55 (2%), 3/55 (6%)	$P < 0.005$ $P < 0.05$ NS NS NS NS	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 61–90 wks King (1988), Imaida *et al.* (1991b)	Intraperitoneal injection *Experiment 1* 0 (control) or 67 µmol/kg bw as 25 µmol/mL in DMSO, 3 ×/wk for 4 wks (total dose, 119 µmol), then observed up to 61 wks Groups of 30, aged 30 d *Experiment 2* 0 (control) or 100 µmol/kg bw in 70 µmol/mL DMSO, once/wk for 4 wks (total dose, 77.3 µmol), then observed up to 87 wks Groups of 30, aged 30 d	*Experiment 1* All tumours: 15/29 (52%), 12/29 (41%) Mammary gland (all tumours): 4/29 (14%), 4/29 (14%) *Experiment 2* All tumours: 26/30 (87%), 25/29 (86%) Mammary gland (all tumours): 11/30 (37%), 17/29 (59%) Mammary gland (fibroadenoma): 9/30 (30%), 14/29 (48%) Mammary gland (adenocarcinoma): 2/30 (7%), 8/29 (28%)*	 NS NS *$P < 0.05$	Purity (NR); mammary tumours were fibroadenomas, adenomas and adenocarcinomas; study limited by short durations of treatment and observation, and use of single doses
CD (F) 76–78 wks King (1988), Imaida *et al.* (1991a)	Intraperitoneal injection 0 (control) or 10 µmol/kg bw as1.7 µmol/mL in DMSO, 3 ×/wk for 4 wks (total dose, 40 µmol/rat) then observed until 76–78 wks Groups of 36 weanling	Mammary gland (all tumours): 7/31 (23%), 25/36 (69%) Mammary gland (fibroadenoma): 5/31 (16%), 19/36 (53%) Mammary gland (adenocarcinoma): 3/31 (10%), 14/36 (39%)	 $P < 0.0001$ $P < 0.001$ $P < 0.01$	Purity, > 99.9%; study limited by short durations of treatment and observation and use of a single dose
F344/DuCrj (M) 72 wks Maeda *et al.* (1986)	Intrapulmonary implantation 0 (control) or 1.50 mg/rat 1-NP or 0.5 mg/rat 3-MC in 0.05 mL beeswax:tricaprylin (1:1) into the lower third of the left lung (× 1) and observed up to 72 wks Groups of 19–32, aged 10–11 wks	Injection site tumours Lung (squamous cell carcinoma): 0/31, 0/32, 19/19 (100%; 3-MC)	 NS for 1-NP	Purity, > 99.9%; study limited by short durations of treatment and observation and use of a single dose; other tumours observed: adrenal gland (4 pheochromo-cytomas, 1 adenoma), pituitary gland (2 adenomas), pancreas (1 islet cell tumour), skin (1 fibroma), leukaemia (1 granulocytic), thymus (1 thymoma)

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 77 wks Imaida *et al.* (1991b)	Direct injection into the mammary gland 0 (control) or 2.03 µmol 1-NP in 0.1 mL DMSO/rat, directly into the 3 left thoracic nipples (d 1) then 3 inguinal nipples (d 2) (total dose 12.3 µmol/rat); inner control: 0.1 mL DMSO injected into the right side of the same rats; then observed until up to 77 wks Groups of 30 aged 30 d	All tumours: 15/28 (53%), 16/27 (59%) Mammary gland (all tumours): 7/28 (25%), 7/27 (26%) Mammary gland (fibroadenoma): 6/28 (21%), 5/27 (18%) Mammary gland (adenocarcinoma): 1/28 (4%), 2/27 (7%)	NS	Purity (NR); tumours other than mammary gland were: 7 pituitary adenomas, 2 pituitary adenocar-cinomas, 2 adrenal cortical adenomas, 1 haemangioma in small intestine

[a] One extraskeletal osteosarcoma and 7 malignant fibrous histiocytomas

[b] Mammary gland tumours, injection site tumours (malignant fibrous histiocytomas) and pituitary, adrenal, thyroid, lung and other tumours at low incidence

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; 3-MC, 3-methylcholanthrene; M, male; 1-NP, 1-nitropyrene; NR, not reported; NS, not significant; wk, week

The incidence of mammary tumours in treated animals (total, 16 out of 35, 46%; fibroadenoma, 9 out of 35, 26%; and adenocarcinoma, 5 out of 35, 14%) did not differ from that in the control group (12 out of 35, 34%; 9 out of 35, 26%; and 5 out of 35, 14%, respectively) (King, 1988; Imaida *et al.*, 1991a).

Groups of 30 female Sprague-Dawley rats, aged 30 days, were administered 0 or 50 µmol of 1-nitropyrene in 0.5 mL of trioctanoin by gavage once a week for 8 weeks (total dose, 400 µmol/rat) and then observed for 41 weeks at which time they were killed. 1-Nitropyrene induced an increased incidence of primarily benign mammary tumours (fibroadenomas) in treated females (10 out of 30, 33%) compared with the controls (0 out of 30; $P = 0.01$) (el-Bayoumy *et al.*, 1995). [The Working Group noted the short durations of treatment and observation.]

3.2.2 Subcutaneous administration

Groups of 20 male Fischer 344/DuCrj rats [age unspecified] received subcutaneous injections of 0 (control) or 20 mg/rat of 1-nitropyrene in 0.2 mL of DMSO twice a week for 10 weeks (total dose, 40 mg). Animals were then observed untreated for an unspecified time until they were killed. The incidence of subcutaneous tumours at the injection site (one extraskeletal osteosarcoma and seven malignant fibrous histiocytomas) was significantly increased compared with controls (0 out of 20 versus 8 out of 17; $P < 0.003$) (Ohgaki *et al.*, 1982). [Ohgaki *et al.* (1985) later reported that the 1-nitropyrene used in this study contained 1,3-dinitropyrene (0.2%), 1,6-dinitropyrene (0.3%) and 1,8-dinitropyrene (0.3%).]

Groups of 28–35 male and 28–35 female newborn Sprague-Dawley-derived CD rats received subcutaneous injections into the suprascapular region of 0 (control), 50 or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO within 24 hours of birth, then once a week for 7 weeks (total of eight injections), and were observed until 62 weeks of age. Rats that survived longer than 113 days (when the first mammary tumour was detected) were included in the effective numbers. The numbers of tumour-bearing rats (all sites) in the control, low-dose and high-dose groups were 6 out of 28 (21%), 10 out of 29 (34%) and 15 out of 31 (48%) males and 8 out of 31 (28%), 19 out of 31 (61%) and 24 out of 32 (75%) females, respectively, with significant increases compared with controls in high-dose males ($P < 0.05$) and low- and high-dose females ($P < 0.01$ and 0.001, respectively). The incidence of malignant fibrous histiocytomas at the injection site was 0 out of 28, 2 out of 29 (7%) and 10 out of 31 (32%) males and 0 out of 31, 3 out of 31 (10%) and 9 out of 32 (28%) females in the control, low-dose and high-dose groups, with significant increases in both high-dose groups ($P < 0.001$). The number of rats with mammary tumours was 2 out of 31 (6%) control, 7 out of 31 (23%) low-dose and 15 out of 32 (47%) high-dose females with a significant increase in the high-dose group ($P < 0.001$). Similarly, the incidence of mammary fibroadenomas (1 out of 31, 3%; 5 out of 31, 16%; and 7 out of 32, 22%) and adenocarcinomas (0 out of 31; 3 out of 31, 10%; and 10 out of 32, 31%) was significantly increased in the high-dose females (7 out of 32 versus 1 out of 31, $P < 0.05$; and 10 out of 32 versus 0 out of 31, $P < 0.01$; respectively) (Hirose *et al.*, 1984).

In a study similar to that of Ohgaki *et al.* (1982), groups of 10 or 20 male Fischer 344/DuCrj rats [age unspecified] received subcutaneous injections of 0 (control), 0.2 or 2.0 mg/rat of 1-nitropyrene (purified by HPLC, with no detectable dinitropyrene impurities: the concentrations of 1,3-dinitropyrene, 1,6- dinitropyrene and 1,8-dinitropyrene were less than 0.05% each, the lowest detectable concentration), dissolved in 0.2 mL of DMSO twice a week for 10 weeks and were then observed for up to 650 days (total doses per rat: 1-nitropyrene, 4 or 40 mg). No subcutaneous sarcomas were observed in the 1-nitropyrene-treated or control rats. The authors concluded that the carcinogenicity of

1-nitropyrene at the injection site observed in the previous study (Ohgaki *et al.*, 1982) was due to contamination from dinitropyrenes and not to 1-nitropyrene itself (Ohgaki *et al.*, 1985).

Groups of 30 female Sprague-Dawley CD rats, aged 30 days, received subcutaneous injections of 0 (control) or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO once a week for 4 weeks (total dose, 74.3 µmol), and surviving rats were killed 90 weeks after the first injection. No difference was found in the incidence of all tumours (27 out of 30 versus 23 out of 29) or all mammary tumours (fibroadenomas and adenocarcinomas combined; 11 out of 30 versus 17 out of 29). However, the incidence of mammary fibroadenomas was significantly increased compared with controls (8 out of 30 versus 15 out of 29; $P < 0.05$) (King, 1988; Imaida *et al.*, 1991b).

Groups of 37–49 female newborn CD 344 rats received subcutaneous injections into the suprascapular region of 0 (control) or 2.5 µmol/kg bw of 1-nitropyrene in DMSO 24 hours after birth, followed by two weekly injections of 0 or 5 µmol/kg bw and five weekly injections of 0 or 10 µmol/kg bw (total dose, 6.3 µmol [18 mg] of 1-nitropyrene in 10 µmol/5.9 mL of DMSO), and were killed at week 67. The incidence of mammary tumours (adenomas, fibroadenomas and adenocarcinomas combined) and adenocarcinomas alone was significantly increased compared with the DMSO controls (8 out of 40 versus 16 out of 49 and 1 out or 40 versus 10 out of 49, respectively) (King, 1988; Imaida *et al.*, 1995).

Groups of 48 or 49 female newborn Sprague-Dawley CD and 55 female newborn Fischer 344 rats received subcutaneous injections into the suprascapular region of 0 (control) or 100 µmol/kg bw of 1-nitropyrene dissolved in DMSO (10 µmol in 5.9 mL) once a week for 8 weeks starting 24 hours after birth and were then observed for up to 86 weeks. In CD rats, the incidence of mammary tumours (adenomas, fibroadenomas and adenocarcinomas combined) and adenocarcinomas was significantly increased compared with the DMSO controls (17 out of 47 versus 26 out of 48 and 3 out of 47 versus 10 out of 48, respectively). In Fischer 344 rats, the incidence of mammary tumours did not different significantly between treated rats and controls (1 out of 55 versus 5 out of 55) (King, 1988; Imaida *et al.*, 1995).

3.2.3 Intraperitoneal injection

Groups of 30 female Sprague-Dawley CD rats, aged 30 days, received intraperitoneal injections of 0 or 67 µmol/kg bw of 1-nitropyrene in 25 µmol/mL of DMSO three times a week for 4 weeks (total dose, 119 µmol), and were then observed up to 61 weeks after the initial injection. The incidence of tumours at all sites and mammary tumours did not differ significantly between treated animals and controls (12 out of 29 versus 15 out of 29 and 4 out of 29 versus 4 out of 29, respectively) (King, 1988; Imaida *et al.*, 1991b).

Groups of 30 female Sprague-Dawley CD rats aged 30 days, received intraperitoneal injections of 0 or 100 µmol/kg bw of 1-nitropyrene in 70 µmol/mL of DMSO once a week for 4 weeks (total dose, 77.3 µmol), and were observed until moribund or up to 87–90 weeks after the initiation of treatment. A significant increase in the incidence of mammary adenocarcinomas was observed (8 out of 29 treated animals versus 2 out of 30 controls; $P < 0.05$) and a non-significant increase in that of fibroadenomas (14 out of 29 treated rats versus 9 out of 30 controls) (King, 1988; Imaida *et al.*, 1991b).

Groups of 36 female weanling Sprague-Dawley CD rats received intraperitoneal injections of 0 or 10 µmol [2.5 mg]/kg bw of 1-nitropyrene (purity, > 99.9%) in 1.7 µmol [0.4 mg]/mL of DMSO three times a week for 4 weeks (total dose, 16 µmol [4 mg]/rat), and were killed when moribund or after 76–78 weeks. The incidence of mammary tumours was significantly increased in treated animals (25 out of 36; 14 adenocarcinomas and 19 fibroadenomas)

Table 3.3 Study of the carcinogenicity of 1-nitropyrene in hamsters

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (M) Yamamoto *et al*. (1987) 663–684 d	Intratracheal instillation 0 (control), 2.0 mg or 2.0 mg benzo[*a*]pyrene (B[*a*]P)/hamster in 0.2 mL phosphate buffer directly into the lung, once/wk for 15 wks (total dose, 30 mg) Groups of 19–35, aged 8 wks	Lung (benign tumours): 0/15, 2/21 (9%), 19/35 (54%; B[*a*]P)	NS for 1-NP	Purity, 98%; impurities (2%): 1,6-DNP, 0.6%; 1,8-DNP, 0.008%; 1,3-DNP, 1.3%; study limited by short duration of treatment, use of a single dose and impurities in the study material

d, day; DNP, dinitropyrene; M, male; 1-NP, 1-nitropyrene; NS, not significant; wk, week

compared with controls (7 out of 31; 3 adenocarcinomas and 5 fibroadenomas; $P < 0.0001$) (King, 1988; Imaida *et al*., 1991a).

3.2.4 Intrapulmonary implantation

Groups of 31 or 32 male Fischer 344/DuCrj rats, aged 10–11 weeks, received a single injection of 0.05 mL beeswax:tricaprylin (1:1) containing 0 or 1.5 mg of 1-nitropyrene (purity, > 99.9%) or 0.5 mg of methylcholanthrene directly into the lower third of the left lung after left lateral thoracotomy. Animals were observed for 72 weeks after treatment, at which time the experiment was terminated. No lung tumours were found in the 1-nitropyrene-treated or control rats, and no difference in the incidence of tumours in other organs was observed among the three groups (Maeda *et al*., 1986). [The Working Group noted that the study was limited by the short durations of both treatment and observation and the use of a single dose.]

3.2.5 Direct injection into the mammary gland

Groups of 30 female CD rats, aged 30 days, received injections of 0 or 2.03 µmol/rat of 1-nitropyrene in 0.1 mL of DMSO directly into the three left thoracic nipple areas (day 1) and then into the three left inguinal nipple areas (day 2) (total dose, 12.3 µmol/rat). As an internal control, 0.1 mL of DMSO was injected into the right-side of the nipple areas of the same rats. Thereafter, the rats were observed for up to 77 weeks. The incidence of all tumours (16 out of 27), total mammary tumours (5 out of 27), fibroadenoma (5 out of 27) and adenocarcinoma (2 out of 27) in the treated group did not differ from that in the controls (15 out of 28, 7 out of 28, 5 out of 28 and 1 out of 28, respectively) (Imaida *et al*., 1991b).

3.3 Hamster

See Table 3.3

3.3.1 Intratracheal instillation

A group of 34 male Syrian hamsters, aged 8 weeks, received intratracheal instillations of 2 mg of 1-nitropyrene (purity, 98%; impurities: 1,3-dinitropyrene, 0.008%; 1,6-dinitropyrene plus 1,8-dinitropyrene, 0.6%; pyrene, 1.3%) suspended in 0.2 mL of phosphate buffer solution once a week for 15 weeks. A further group of 35 hamsters received instillations of 2 mg of benzo[*a*]pyrene and a control group of 19 animals received phosphate buffer solution alone. All hamsters in the 1-nitropyrene-treated and control groups died within 663 and 684 days, respectively, after the initial instillation; after the 15 instillations, 24 and 16 animals in these groups, respectively,

were still alive (some died from pneumonia). Lung adenomas were detected in 2 out of 21 (9%) 1-nitropyrene-treated animals, one of which also had a squamous cell papilloma of the trachea. Tumours of the respiratory organs occurred in 19 out of 22 (54%) benzo[*a*]pyrene-treated hamsters, but not in controls (Yamamoto *et al.*, 1987). [The Working Group noted that the study was limited by the short duration of treatment, the use of a single dose level and dinitropyrene impurities in the material studied.]

4. Mechanistic and Other Relevant Data

1-Nitropyrene is a constituent of many combustion emissions, especially diesel exhaust, and has been used as a marker in air for occupational exposure to diesel exhaust. 1-Nitropyrene has been reviewed previously (IARC, 1989; IPCS, 2003); additional studies were reviewed by the Working Group.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No studies were available to the Working Group on 1-nitropyrene alone, which forms part of complex mixtures – typically diesel exhaust and ambient air contaminated with diesel exhaust – to which humans are exposed. Nevertheless, several studies have shown greater concentrations of 1-nitropyrene in diesel exhaust-contaminated air or increased urinary metabolites of 1-nitropyrene in people working in such atmospheres, suggesting that this compound may be a suitable marker for exposure to diesel exhaust in the atmosphere (Scheepers *et al.*, 1995a).

Five metabolites of 1-nitropyrene were found in the urine samples from 17 men and five women living in the city of Kanazawa, Japan (for whom no occupations associated with diesel exhaust emissions or smoking status were reported) (see Section 1.5.1(*b*)), using liquid chromatography-MS/MS, were mostly glucuronides or sulfate conjugates: 117, 109, 203, 137 and ≤ 0.54 pmol/mol of creatinine of 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene, 6- and 8-hydroxy-1-nitropyrene and 3-hydroxy-1-nitropyrene, respectively (Toriba *et al.*, 2007; see Fig. 4.1).

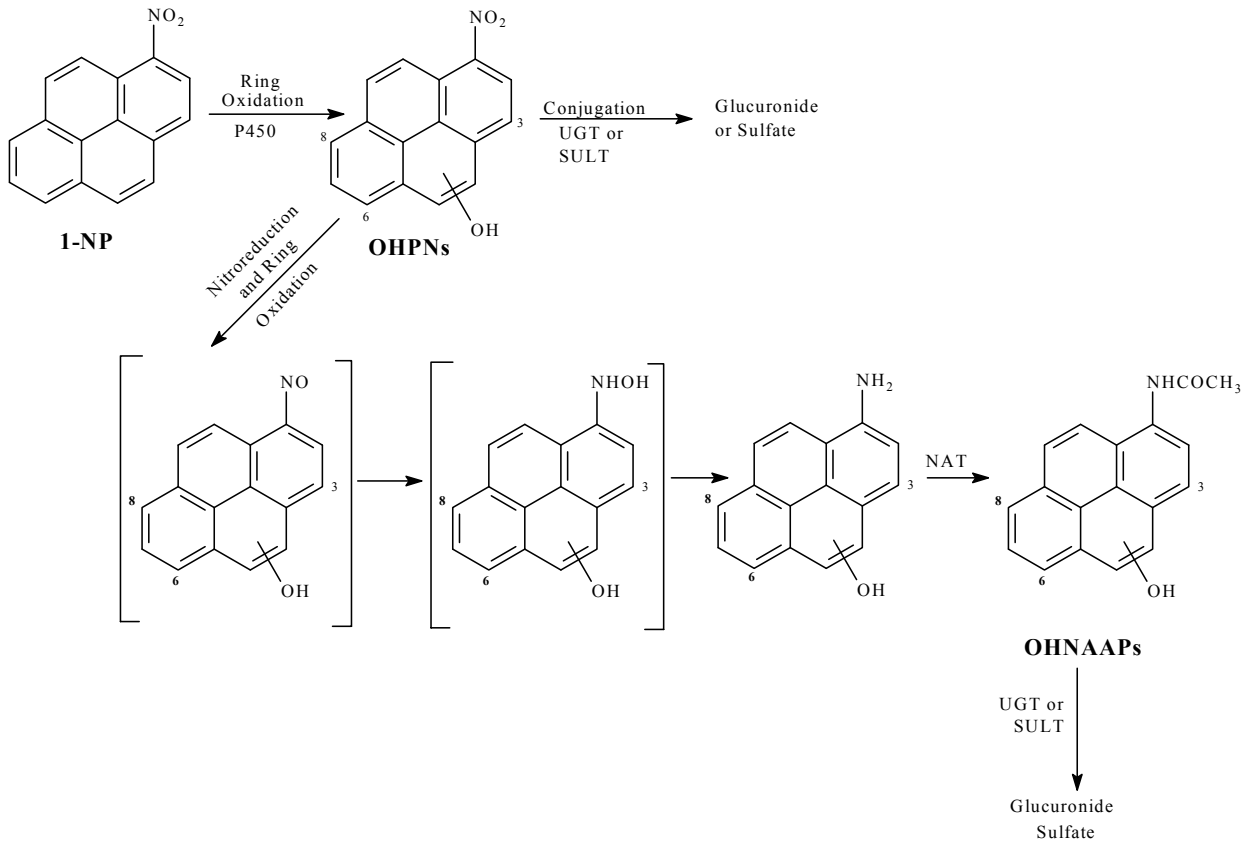
Scheepers *et al.* (1995a) found higher concentrations of 1-nitropyrene in the air of a workshop of diesel bus mechanics than in office air, and showed that the concentration was one order of magnitude greater in the shop during work hours than during off-work hours. Similarly, concentrations of 1-nitropyrene were higher in the air of a repair shop for diesel train engines than in office air, and a similar correlation was found for urinary metabolites of 1-nitropyrene in the workers (Scheepers *et al.*, 1994b). High levels of urinary metabolites of 1-nitropyrene have also been found in salt miners using diesel equipment underground (Seidel *et al.*, 2002).

4.1.2 Experimental systems

Since the previous review of 1-nitropyrene (IARC, 1989), additional studies have clarified its kinetics and metabolism, especially with regard to the role of microflora in and the organ specificity of its metabolism. A large number of studies have combined aspects of metabolism, particularly the role of nitroreductase, with genotoxicity end-points, and these are reviewed in Section 4.2.2.

Howard *et al.* (1995) found that administration of 1.03 mg/kg of [4,5,9,10-^{3}H]1-nitropyrene to C57B1/6N mice by gavage resulted in biphasic elimination kinetics from the blood with half-lives of 0.3 and 1.8 days; intraperitoneal administration resulted in similar biphasic elimination but with half-lives of 0.5 and 3 days. They also showed that, after administration of 1 mg/kg of

Fig. 4.1 Major metabolites of 1-nitropyrene in human urine



NAT, *N*-acetyltransferase; 1-NP, 1-nitropyrene; OHNAAPs, hydroxy-*N*-acetyl-1-aminopyrenes (6- and 8-OHNAAP); OHNPs, hydroxy-1-nitropyrenes (3-, 6-, and 8-OHNP); P450, cytochrome P450; SULT, sulfotransferase; UGT, UDP-glucuronyltransferase
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[4,5,9,10-^{3}H]1-nitropyrene by gavage to pregnant mice, 1-nitropyrene crossed the placenta and accumulated in the fetus and amniotic fluid; both carbon oxidized and nitroreduced metabolites were formed. The neonates received ~0.1% of the administered dose and harboured both types of metabolite.

Wolff *et al.* (1989) noted that 1-nitropyrene is typically associated with carbonaceous particles in the environment rather than as an aerosol of the pure compound, and showed that, when [^{14}C]1-nitropyrene adsorbed to carbon black particles was inhaled by Fischer 344 rats, the interaction of the reactive metabolites of [^{14}C]1-nitropyrene with target macromolecules was greater than that caused by inhalation of [^{14}C]1-nitropyrene as a pure compound.

Using a DNA repair-deficient Chinese hamster ovary cell line, Thornton-Manning *et al.* (1991a) tested the mutagenicity of 1-nitropyrene under aerobic and anaerobic metabolic conditions and analysed the metabolites. The results suggested that the exogenous metabolic activation-mediated metabolites produced under anaerobic conditions resulted from nitroreductive metabolism (1-aminopyrene), whereas those produced under aerobic conditions of exogenous metabolic activation-mediated metabolism resulted from ring-oxidized metabolites (1-nitropyrene phenols and dihydrodiols).

The treatment of HepG2 cells with 1-nitropyrene, followed by administration of 3-methylcholanthrene, increased the ratio of ring-oxidation to nitroreduction, which was accompanied by a decrease in the C-8-guanyl adduct of 1-nitropyrene (*N*-(deoxyguanosine-8-yl)-1-aminopyrene; dG-C8-AP) via nitroreduction with no further increase in other 1-nitropyrene adducts (Silvers *et al.*, 1994). The authors suggested that the cytochrome P450 (CYP)-mediated metabolism of 1-nitropyrene to epoxides, phenols and dihydrodiols is not an activation pathway in HepG2 cells, and indicated that this might explain the weak carcinogenicity 1-nitropyrene *in vivo*, in which CYP-mediated ring-oxidation predominates.

Van Bekkum *et al.* (1999) administered [^{14}C]-1-nitropyrene intragastrically to rats and found that it was absorbed rapidly from the gastrointestinal tract based on early peak concentrations of radioactivity in blood and other tissues. These data indicated an important role of intestinal microflora in the enterohepatic recirculation but not in the nitroreduction of 1-nitropyrene before adsorption from the gastrointestinal tract. The levels of radioactivity associated with plasma proteins were approximately four times higher than those associated with haemoglobin, and those associated with DNA were highest initially in the liver but decreased rapidly to levels lower than those observed in the kidney. The radioactivity associated with DNA in the lungs was 8–50 times lower than that in the liver and kidneys.

Nitroreductase activity, measured by the production of 1-aminopyrene from 1-nitropyrene, following treatment with various organ homogenates from Sprague-Dawley rats was found to be high in the liver and small intestine but low in the lung and alveolar macrophages (Kinouchi & Ohnishi, 1986). The intestinal contents also had high nitroreductase activity that was proportional to the number of bacteria (especially anaerobic bacteria) in the intestine, which provided further evidence of the potential importance of the gut flora in the metabolism of 1-nitropyrene via nitroreduction.

To examine this further, Kinouchi *et al.* (1992, 1993) pretreated rats with antibiotics to kill the gut flora *in vivo* followed by oral administration of K-region epoxides of 1-nitropyrene [1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide] and found no DNA adducts in the lower intestinal mucosa, whereas adducts were found in unpretreated rats exposed to these conjugates. The authors showed that the intestinal microflora played an important role in the adsorption of the metabolites of glutathione conjugates of 1-nitropyrene oxides from the intestinal tract and the activation of the metabolites in the intestine. Kinouchi *et al.* (1993) also showed that DNA adducts were formed in the intestinal mucosa after administration of glutathione conjugates of 1-nitropyrene oxides (1-nitropyrene-4,5-oxide *S*-glutathione and 1-nitropyrene-9,10-oxide *S*-glutathione) to control mice, which indicated the metabolic activation of cysteine conjugates of 1-nitropyrene oxides (1-nitropyrene oxide-cysteine) by microfloral β-lyase. Kataoka *et al.* (1995) also showed that 1-nitropyrene oxide-cysteine is converted to a more genotoxic form by β-lyase-mediated deconjugation and nitroreduction.

Ball *et al.* (1991) administered [^{14}C]1-nitropyrene intraperitoneally to germ-free and conventional rats and showed that formation of the metabolite, 6-hydroxy-1-acetylaminopyrene, in the urine involved the gut flora; both nitroreduction and the hydrolysis of glucuronides released for enterohepatic recirculation were essential for the generation of the mutagenic urinary metabolites of 1-nitropyrene.

However, Ayres *et al.* (1985) demonstrated that gut microfloral metabolism was not the only pathway for the bioactivation of 1-nitropyrene in rats, because the level of macromolecular binding of radiolabelled 1-nitropyrene administered orally to rats was initially reduced

by almost half in the lungs of animals pretreated with antibiotics to kill the gut flora, compared with unpretreated rats, although the levels were similar 1 week later.

In an in-vitro study, King *et al.* (1990) found that 1-nitropyrene was metabolized by human, rat or mouse intestinal microflora to 1-aminopyrene, *N*-acetyl-1-aminopyrene, *N*-formyl-1-aminopyrene and two unknown metabolites. 1-Aminopyrene was the predominant metabolite generated by all three microflora, accounting for 98, 79 and 88% of the total radioactivity, respectively. Thus, microflora from all of the species that were tested metabolized 1-nitropyrene in a similar manner.

Rafii & Cerniglia (1995) identified and isolated various species of *Clostridium* and *Eubacterium* from human intestinal microbial flora that metabolized 1-nitropyrene *in vitro* to produce aromatic amines. The nitroreductase activities of the bacteria were found to be constitutive and extracellular and, although each bacterial species had one isozyme, each species had different isozymes.

Studies in rodents involving intragastric, intraperitoneal or inhalation routes of exposure to 1-nitropyrene or 1-nitropyrene coated onto diesel exhaust particles found that the majority (50–60%) of the administered dose was excreted in the faeces and ~15–20% appeared in the urine (Bond *et al.*, 1986; IARC, 1989; Howard *et al.*, 1990; Kataoka *et al.*, 1991; Silvers *et al.*, 1992). As reviewed by van Bekkum *et al.* (1999), the major pathways in the biotransformation of 1-nitropyrene administered to rats, on the basis of the metabolites found in the urine and faeces, are nitroreduction with subsequent acetylation or *N*-oxidation, ring-hydroxylation and conjugation. After intraperitoneal administration of 1-nitropyrene to rats, Chae *et al.* (1997) identified metabolites in the urine and faeces that had been formed via nitroreduction and ring-oxidation pathways.

A comparison of the macromolecular binding of radiolabelled 1-nitropyrene following its oral administration to or inhalation by rats showed that the greatest amount of binding occurred in the kidney, followed by the liver and then the lung, regardless of the route of administration (Medinsky *et al.*, 1988).

Coal fly ash particles (≤ 3 μm) were coated with 1-nitropyrene by exposure to vapour in a nitrogen atmosphere. Extraction with organic solvents and chemical analysis showed that the concentration of 1-nitropyrene was 160–220 ppm. 1-Nitropyrene was shown to be bioavailable in rabbit alveolar macrophages exposed to this coated fly ash in either agar or aqueous culture medium. The coated fly ash showed mutagenic activity when the particles were tested directly, whereas the uncoated fly ash did not (Mumford *et al.*, 1986).

Rosser *et al.* (1996) proposed a generalized scheme for the metabolism of 1-nitropyrene in rodents that involves nitroreduction, epoxidation, enhancement of the genotoxicity of a hydroxylamine intermediate by *O*-acetylation, and further activation of an arylacetamide. They suggested that *N*-hydroxylation followed by *O*-esterification, as opposed to further exogenous metabolic activation-catalysed ring-oxidation, is a major route of activation for the urinary metabolites of 1-nitropyrene in rodents.

Using human lung and liver microsomes, Chae *et al.* (1999) identified some differences between the metabolism of 1-nitropyrene in humans and rodents. They suggested that human liver does not metabolize 1-nitropyrene to 1-aminopyrene, whereas rodent liver does. They also showed that CYP3A4, and to a lesser extent CYP1A2, produced 3-hydroxy-1-nitropyrene as the major hydroxylated metabolite in humans, whereas 6-hydroxy- and 8-hydroxy-1-nitropyrene were produced at lower levels. In contrast, the 6- and 8-hydroxy metabolites were produced to a greater extent than 3-hydroxy-1-nitropyrene in rodents. The authors also showed

that *trans*-4,5-dihydrodiol-1-nitropyrene was produced preferentially to the glutathione conjugate in humans, whereas the reverse occurred in rodents. The authors concluded that, due these metabolic differences, rodents may not accurately predict the susceptibility of humans to 1-nitropyrene-induced carcinogenicity. Sun *et al.* (2004) found that primary cultures of human breast cells and cultured human breast cell lines activated 1-nitropyrene to genotoxic metabolites via ring-oxidation.

Using sensitive analytical techniques, Toriba *et al.* (2007) analysed the urine from healthy, non-occupationally exposed subjects and identified various 1-nitropyrene metabolites, including 6- and 8-hydroxy-*N*-acetyl-1-aminopyrenes and 3-, 6- and 8-hydroxy-1-nitropyrenes. These metabolites had been predicted to occur in human urine on the basis of in-vitro and rodent studies of the metabolism of 1-nitropyrene.

Ueda *et al.* (2005) also showed that molybdenum hydroxylases from the skin of mice, rats and guinea-pigs were involved in the nitroreduction of 1-nitropyrene.

Probably due to differences in metabolism, 1-nitropyrene produces tumours at different organ sites in different species: lung and liver cancer in mice, tracheal cancer in hamsters and mammary cancer in rats (see Chae *et al.*, 1999).

4.2 Genetic and related effects

See Table 4.1

4.2.1 Humans

No studies were available to the Working Group in which humans were exposed solely to 1-nitropyrene. However, studies have been carried out in humans exposed to complex combustion emissions (especially diesel exhaust) containing 1-nitropyrene, and these are reviewed in Section 4.2.1 of the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume.

4.2.2 Experimental systems

(a) Formation of DNA adducts

1-Nitropyrene induced DNA adducts in a variety of systems, and additional studies reviewed below have confirmed and extended the initial observations described in the previous *Monograph* (IARC, 1989).

(i) In-vivo studies

See Fig. 4.2

Administration of 1-nitropyrene (100 mg/kg bw) by gavage to rats produced DNA adducts detected by ^{32}P-postlabelling in the liver and mammary fat pads (Roy *et al.*, 1989). In the same study, the authors exposed calf-thymus DNA to 1-nitropyrene in the presence of xanthine and xanthine oxidase and confirmed the formation of the adduct dG-C8-AP, which co-chromatographed with a minor adduct found in the liver and mammary fat pads. The authors concluded that the major adduct formed *in vivo* did not seem to originate from the simple nitroreduction of 1-nitropyrene and that other pathways, such as ring-oxidation or ring-oxidation followed by nitroreduction, may account for the major adduct found *in vivo*. However, Chae *et al.* (1997) found no DNA adducts in the liver but did find adducts in mammary DNA in rats after intraperitoneal injection of 1-nitropyrene.

Herreno-Saenz *et al.* (1995) also found the primary dG-C8-AP DNA adduct in the mammary tissue of rats exposed to 1-nitropyrene [dose not reported], and also identified two minor adducts: 6-(deoxyguanosin-N2-yl)-1-aminopyrene and 8-(deoxyguanosin-N2-yl)-1-aminopyrene. Smith *et al.* (1990) identified dG-C8-AP and *N*-(deoxyguanosin-8-yl)-1-amino-3-, 6- and/or 8-nitropyrene DNA adducts in Sprague-Dawley rats, and CD-1 and A/J mice.

Haemoglobin and albumin adducts were identified in rats administered 1-nitropyrene (dose range, 0.1–1000 µg/kg bw) by gavage (el-Bayoumy *et al.*, 1994a, b), and another study

Table 4.1 Genetic and related effects of 1-nitropyrene

Reference	System	Result
DNA adducts *in vivo*		
Roy *et al.* (1989)	Rat, gavage; mammary fat pads and liver	+
Chae *et al.* (1997)	Rat, intraperitoneal; mammary tissue	+
Chae *et al.* (1997)	Rat, intraperitoneal; liver	–
Herreno-Saenz *et al.* (1995)	Rat, gavage; mammary tissue	+
Smith *et al.* (1990)	Rat and mouse, subcutaneous or intraperitoneal; mammary gland and site of injection (rat), liver and lung (mouse)	+
el-Bayoumy *et al.* (1994b)	Rat, gavage	+
DNA adducts *in vitro*		
Arimochi *et al.* (1998)	*Salmonella typhimurium*	+
Herreno-Saenz *et al.* (1995)	*Salmonella typhimurium*	+
Herreno-Saenz *et al.* (1995)	Calf-thymus DNA plus xanthine oxidase or rat liver microsomes and cytosol	+
Roy *et al.* (1989)	Calf-thymus DNA plus xanthine oxidase	+
King *et al.* (1994)	Calf-thymus DNA	+
Mitchell & Akkaraju (1989)	Rat lung-cell nuclei	+
Kucab *et al.* (2012)	Hupki (human *TP53* knock-in) mouse embryo fibroblasts (J201)	+
Silvers *et al.* (1997)	HepG2 cells	+
Qu & Stacey (1996)	Rat hepatocytes	+
Haemoglobin adducts *in vivo*		
van Bekkum *et al.* (1997)	Rat, gavage	+
el-Bayoumy *et al.* (1994b)	Rat, gavage	+
Suzuki *et al.* (1989)	Rat, gavage	+
Albumin adducts *in vivo*		
el-Bayoumy *et al.* (1994a)	Rat, gavage	+
DNA damage *in vivo*		
el-Bayoumy *et al.* (2000)	Rat, gavage; 8-OH-dG in mammary fat pad	–
Igarashi *et al.* (2010)	Mouse, intraperitoneal; comet assay, liver	+
DNA damage *in vitro*		
Becher *et al.* (1993)	Rabbit lung cells (Clara and type II) and alveolar macrophages; alkaline elution	–
Andersson *et al.* (2009)	Human umbilical vein endothelial cells; comet assay	+
Martin *et al.* (1999)	Human breast cancer cell line (MCL-5); comet assay	–
Martin *et al.* (2000)	Exfoliated human breast milk cells; comet assay	+
Kucab *et al.* (2012)	Hupki (human *TP53* knock-in) mouse embryo fibroblasts (J201); comet assay	+

Table 4.1 (continued)

Reference	System	Result
Mitchelmore *et al.* (1998)	Digestive gland cells from mussels; comet assay	+
Mori *et al.* (1991)	Rat primary hepatocytes; tritium	+
Kim *et al.* (2005b)	Human lung cell line A549; 8-OH-dG	+
Asare *et al.* (2009)	Mouse Hepa1c1c7 cells; comet assay, formamidopyrimidine-DNA glycosylase, 8-oxoguanine	+
Li *et al.* (2009)	Human lymphocytes; comet assay	+
Schehrer *et al.* (2000)	Rat and human hepatocytes; unscheduled DNA synthesis	+
Gene mutation in bacteria		
Hakura *et al.* (1999)	*Salmonella typhimurium* TA98 with human or rat S9	+
Hatanaka *et al.* (2001)	*Salmonella umu* assay with rat lung, liver, kidney S9	+
DeMarini *et al.* (1989)	*Salmonella typhimurium* TA98 micro-suspension assay	+
Lewtas *et al.* (1990)	*Salmonella typhimurium* TM677, TA98 coupled to HPLC	+
Yu *et al.* (1991)	*Salmonella typhimurium* TA98, TA97, TA98, TA98NR, TA98/1,8-DNP6, TA100, TA100NR, TA102, TA104, TA1538	+
Salamanca-Pinzón *et al.* (2006, 2010)	*Salmonella typhimurium*, enzyme extracts of nitroreductases	+
Watanabe *et al.* (1989)	*Salmonella typhimurium* YG1021	+
Oda *et al.* (1992)	*Salmonella typhimurium* NM1011	+
Oda *et al.* (1993)	*Salmonella typhimurium* NM3009	+
Carroll *et al.* (2002)	*Salmonella typhimurium* TA1535, TA 1538, TA100, TA98	+
Østergaard *et al.* (2007)	*Salmonella typhimurium* TGO1, TGO2	+
Consolo *et al.* (1989)	*Salmonella typhimurium* TA98, TA98NR, TA98/1,8DNP6	+
Arimochi *et al.* (1998)	*Salmonella typhimurium* TA98, TA98NR, YG1021	+
Hagiwara *et al.* (1993)	*Salmonella typhimurium* YG1021, YG1024, YG1041	+
Watanabe *et al.* (1993)	*Salmonella typhimurium* YG1024, YG1012	+
Watanabe *et al.* (1990)	*Salmonella typhimurium* YG1024, YG1029	+
Oda *et al.* (1999)	*Salmonella typhimurium* NM6001, NM6002	+
Mersch-Sundermann *et al.* (1991)	*Escherichia coli* PQ37, SOS Chromotest; –S9	+
Rosser *et al.* (1996)	*Salmonella typhimurium* YG1024, TA98, TA98NR, TA98/1,8-DNP6	+
Yamazaki *et al.* (2000)	*Escherichia coli* expressing human CYP1B1	+
Oda *et al.* (1996)	*Salmonella typhimurium* NM5004 expressing rat GST T1	w+
Oda *et al.* (2012)	*Salmonella typhimurium* expressing human CYP1A2/sulfotransferase	+
Bonnefoy *et al.* (2012)	*Salmonella umu* assay ± S9	+/+

Table 4.1 (continued)

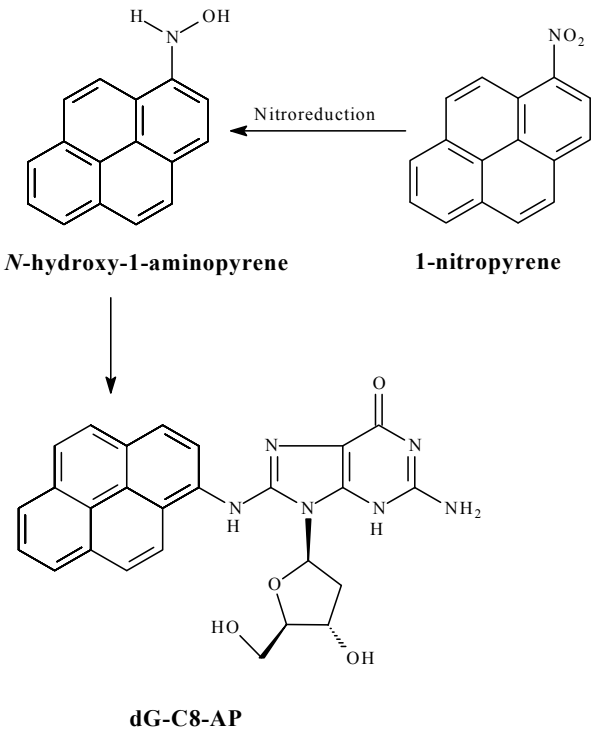
Reference	System	Result
Watanabe *et al.* (1997)	*Salmonella typhimurium* TA7005, TA7006, related strains; –S9	+
DeMarini *et al.* (1996)	*Salmonella typhimurium* UTH8413, TA1538, TA98, TA100 ; –S9	+
DeMarini *et al.* (1996)	*Salmonella typhimurium* TA1978, TA1975, UTH8414, TA1535 ; –S9	–
Watanabe-Akanuma & Ohta (1994)	*Escherichia coli lacZ* ZA1607, 08, 10, 11; ZA8108, 10, 11; –S9	+
Nohmi *et al.* (1995)	*Salmonella umuDCST* required for –GC mutagenesis,–-S9	+
Gene mutation in mammalian cells *in vivo*		
Ball *et al.* (1991)	Rat, germ-free, intraperitoneal; urinary mutagenicity in *Salmonella typhimurium* TA98 ± S9	w+
Ball *et al.* (1991)	Rat, conventional, intraperitoneal; urinary mutagenicity in *Salmonella typhimurium* TA98 ± S9	+
Varga *et al.* (2006)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA100	+
Varga *et al.* (2006)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA98	–
Varga *et al.* (2006)	Rat, gavage; urinary mutagenicity *Salmonella typhimurium* TA98, TA100	+
Scheepers *et al.* (1991)	Rat, intraperitoneal; urinary mutagenicity *Salmonella typhimurium* TA98, TA1538, YG102, YG1024	+
Gene mutation in mammalian cells *in vitro*		
Rodent cells		
Heflich *et al.* (1990)	CHO/*Hprt* –S9	–
Heflich *et al.* (1990)	CHO/*Hprt* +S9	+
Thornton-Manning *et al.* (1991a, b)	CHO-UV5/*Hprt* (repair-deficient) –S9	+
Kappers *et al.* (2000)	Chinese hamster V79/*Hprt* expressing CYP1A2 or CYP3A4 –S9	–
Kappers *et al.* (2000)	NIH/3T3/*lacZ* reporter gene, expressing CYP1A2 –S9	+
	Human cells	
Silvers *et al.* (1994)	HepG2/*HPRT* –S9	+
Durant *et al.* (1996)	Human B-lymphoblastoid h1A1vs/*HPRT* expressing CYP1A1 –S9	+
Gene mutation in yeast		
Rhenimi *et al.* (2008)	Gene mutation, gene conversion, crossing-over	+
Gene mutation in plants		
Katoh *et al.* (1994)	Soya bean, gene mutation	+
Cytogenetic effects		
Chromosomal aberrations		
Lafi & Parry (1987)	Chinese hamster DON:Wg3H cells, –S9	+
Matsuoka *et al.* (1991)	Chinese hamster lung cells, –S9	–
Matsuoka *et al.* (1991)	Chinese hamster lung cells, +S9	+

Table 4.1 (continued)

Reference	System	Result
Pusztai *et al.* (1998)	Mouse, intraperitoneal; bone marrow *in vivo*	+
Micronuclei		
Bonnefoy *et al.* (2012)	Human lymphocytes, 63% no centromere (chromatid breakage)	+
Igarashi *et al.* (2010)	Mouse, intraperitoneal; liver *in vivo*	+
Transformation		
West & Rowland (1994)	Rat tracheal epithelial cells *in vitro*	-
Ensell *et al.* (1998)	Rat tracheal epithelial cells *in vitro*	-
Mitchell & Thomassen (1990)	Rat tracheal epithelial cells *in vitro*	-
Ensell *et al.* (1998, 1999)	Rat tracheal epithelial cells *in vivo*	+
Sheu *et al.* (1994)	BALB/3T3 A31-1-1 cells *in vitro*	+
Denda *et al.* (1989)	F344 rat liver, γ-glutamyl transpeptidase-positive foci *in vivo*	+

CHO, Chinese hamster ovary cells; CYP, cytochrome P450; GST, glutathione *S*-transferase; Hprt/HPRT, hypoxanthine-guanine phosphoribosyltransferase; 8-OH-dG, 8-hydroxydeoxyguanosine; S9, exogenous metabolic activation system

Fig. 4.2 The major DNA adduct identified *in vivo* in animals treated with 1-nitropyrene



dG-C8-AP, *N*-(deoxyguanosin-8-yl)-1-aminopyrene

found that the levels of haemoglobin adducts were twofold higher than those of albumin adducts in rats after oral administration of 1-nitropyrene (van Bekkum *et al.*, 1997). Suzuki *et al.* (1989) also identified haemoglobin adducts in rats exposed to 1-nitropyrene *in vivo*.

(ii) In-vitro studies

Arimochi *et al.* (1998) exposed *S. typhimurium* strains that had three levels of nitroreductase activity (low, wild-type and excessive) to 1-nitropyrene and showed that the levels of DNA adducts and mutagenicity correlated with those of nitroreductase.

The dG-C8-AP adduct was found in 1-nitropyrene-treated calf-thymus DNA in the presence of xanthine and xanthine oxidase, as well as two minor adducts: 6-(deoxyguanosin-N2-yl)-1-aminopyrene and 8-(deoxyguanosin-N2-yl)-1-aminopyrene. All three adducts were also found in 1-nitropyrene-treated calf-thymus DNA in the presence of rat liver microsomes and cytosols and in 1-nitropyrene-treated DNA from *Salmonella* (Herreno-Saenz *et al.*, 1995). The dG-C8-AP adduct was also formed after exposure of calf-thymus DNA to 1-nitropyrene in the presence of xanthine oxidase (Roy *et al.*, 1989).

Another study of 1-nitropyrene-exposed calf-thymus DNA found a variety of DNA adducts determined by HPLC-^{32}P postlabelling; however, none of these peaks co-eluted with those produced by the incubation of calf-thymus DNA with an organic solvent extract of diesel exhaust particles (King *et al.*, 1994).

Mitchell & Akkaraju (1989) exposed rat lung cell nuclei *in vitro* to radiolabelled 1-nitropyrene and found that more of the label was bound to active chromatin and the nuclear matrix than to bulk chromatin fractions. They concluded that the selective binding of 1-nitropyrene to specific regions was related to the open state of the chromatin structure.

1-Nitropyrene induced DNA adducts (detected by ^{32}P-postlabelling) to a greater extent in an immortal Hupki (human *TP53* knock-in) mouse embryo fibroblast (HUF) cell line (J201) than in primary Hupki (human *TP53* knock-in) mouse embryo fibroblasts (Kucab *et al.*, 2012). 1-Nitropyrene also induced DNA adducts (detected by postlabelling) in freshly isolated rat hepatocytes (Qu & Stacey, 1996).

Exposure of a HepG2 human hepatoblastoma cell line to 1-nitropyrene resulted in the formation of the dG-C8-AP adduct, and the induction of ring-oxidative metabolism by treatment with 2,3,7,8-tetrachlorodibenzodioxin resulted in a decrease in the levels of this adduct (Silvers *et al.*, 1997). The authors concluded that CYP-mediated ring-oxidative pathways were detoxification pathways in HepG2 cells because no DNA adducts of oxidized 1-nitropyrene metabolites were detected in the 2,3,7,8-tetrachlorodibenzodioxin-treated cells exposed to

1-nitropyrene. The authors also exposed HepG2 cells to 1-nitropyrene and 3-methylcholanthrene to increase the ratio of ring-oxidation to nitroreduction and found a decrease in the dG-C8-AP adduct (via nitroreduction) with no increase in any other 1-nitropyrene DNA adducts.

(b) DNA damage

8-Hydroxy-2′-deoxyguanosine, a marker of oxidative damage, was not found in mammary fat pad tissue isolated from rats administered 1-nitropyrene by gavage (el-Bayoumy *et al.*, 2000). Intraperitoneal administration of 1-nitropyrene to mice induced DNA damage in the liver detected by the comet assay (Igarashi *et al.*, 2010).

Using alkaline elution, Becher *et al.* (1993) did not find any DNA damage in isolated rabbit lung cells (Clara and type II) or lung alveolar macrophages exposed to 1-nitropyrene *in vitro*. No DNA damage was found in the comet assay in 1-niropyrene-treated human breast cancer cells (MCL-5) *in vitro*, even in the presence of DNA-repair inhibitors (Martin *et al.*, 1999).

The induction of DNA damage was demonstrated in the comet assay in other cell types exposed to 1-nitropyrene *in vitro*, including human umbilical vein endothelial cells (Andersson *et al.*, 2009), exfoliated human breast milk cells in the presence of DNA-repair inhibitors (Martin *et al.*, 2000), Hupki (human *TP53* knock-in) mouse embryo fibroblasts (Kucab *et al.*, 2012), mouse Hepa1c1c cells (Asare *et al.*, 2009), human lymphocytes (Li *et al.*, 2009) and digestive gland cells isolated from mussels (Mitchelmore *et al.*, 1998). The use of tritium to monitor unscheduled DNA synthesis also showed that 1-nitropyrene induced DNA damage in primary rat hepatocytes *in vitro* (Mori *et al.*, 1991).

1-Nitropyrene also has been shown to induce oxidative damage, as demonstrated by the presence of 8-hydroxy-2′-deoxyguanosine, after in-vitro treatment of the A549 human lung adenocarcinoma cell line (Kim *et al.*, 2005a). It induced DNA damage, as detected in the unscheduled DNA synthesis assay, in human and rat hepatocytes (Schehrer *et al.*, 2000).

(c) Mutagenicity

1-Nitropyrene was mutagenic in a variety of systems, as reviewed in the previous *Monograph* (IARC, 1989). Additional studies that have explored the metabolism required to produce mutagenic metabolites of 1-nitropyrene, its mutation spectrum and the molecular mechanisms by which it induces mutations are reviewed below.

(i) Bacterial mutagenesis

Hayakawa *et al.* (1997) fractionated organic extracts of diesel engine exhaust particles and showed that 1-nitropyrene, together with several other nitro-PAHs, accounted for half of the mutagenic activity of the semi-polar fraction of diesel exhaust particles. Thus, 1-nitropyrene is a highly relevant component of diesel exhaust in terms of the mutagenicity of this complex combustion emission. Although the mechanisms of the interactions of 1-nitropyrene with other components of diesel exhaust emissions have not been elucidated, Lee *et al.* (1994) showed that, when benzo[*a*]pyrene in combination with 1-nitropyrene was tested for mutagenicity in *Salmonella* in the absence of an exogenous metabolic activation system, the mutagenic potency of 1-nitropyrene was reduced, and presumed that this was due to alterations in the nitroreductase activity of the bacterium.

Hakura *et al.* (1999) demonstrated that the mutagenic potency of 1-nitropyrene in *S. typhimurium* TA98 in the presence of five exogenous metabolic activation systems produced from the livers of different human subjects was similar to or slightly greater than that in the presence of a system produced from non-induced Sprague-Dawley rat liver. Because 1-nitropyrene is one of the primary mutagens in diesel exhaust, Hatanaka *et al.* (2001) exposed rats to 0.3 mg/m^3 of diesel exhaust particles and found that the level of induction of CYP1B1 was sufficient for

the exogenous metabolic activation systems produced from their lungs, liver or kidneys to metabolize 1-nitropyrene to a genotoxic agent in the *Salmonella umu* gene-expression assay in a strain that overexpressed *O*-acetyltransferase.

1-Nitropyrene was mutagenic in a micro-suspension assay in *Salmonella* (DeMarini *et al.*, 1989), which was then used to detect 1-nitropyrene metabolites generated by exogenous metabolic activation systems (Lewtas *et al.*, 1990), by incubating 1-nitropyrene with a system from rabbit lung and characterizing the mutational profile of the metabolites that were separated by bioassay-directed fractionation using HPLC and then tested in this assay. The HPLC fractions were tested in forward mutation assays in *S. typhimurium* strains TM677 and TA98. Approximately 12 peaks of mutagenic activity were detectable by TA98, suggesting that at least the same number of mutagenic metabolites were formed from the metabolism of 1-nitropyrene by the rabbit lung activation system. Six of the peaks were identified using standards that co-chromatographed with the peaks of mutagenic activity in the 'mutagram': 3- or 8-hydroxy-*N*-acetyl-l-aminopyrene, [*trans*]4,5-dihydro-4,5-dihydroxy-l-nitropyrene, *N*-acetyl-1-aminopyrene, 1-aminopyrene and 3-, 6- or 8-hydroxy-nitropyrene.

1-Nitropyrene was found to be a direct-acting mutagen in a wide variety of *S. typhimurium* strains, including TA98, TA97, TA98, TA98NR, TA98/1,8-DNP_6, TA100, TA100NR, TA102, TA104, TA1035 and TA1538 (Yu *et al.*, 1991). It was most potent in TA97, which contains a series of six cytosines and reverts by the frameshift deletion of a C in this sequence. This result was consistent with the formation of 1-nitropyrene adducts at GC sites and with the known frameshift activity of 1-nitropyrene in TA98, which contains repeated series of GCs. This study confirmed that the lower levels of nitroreductase present in TA98NR reduced the mutagenic potency of 1-nitropyrene relative to that in TA98. It also showed that 1-nitropyrene was a potent mutagen in strain TA104, which reverts by mutation at AT sites, indicating that the compound also forms adenine adducts. Several studies have indicated that the *cnr* gene in *Salmonella* codes for the major nitroreductase responsible for activating 1-nitropyrene to a mutagen (Salamanca-Pinzón *et al.*, 2006, 2010).

Whereas *S. typhimurium* strains TA98NR or TA100NR, which are deficient in nitroreductase, reduced the mutagenic potency of 1-nitropyrene compared with that in the parent strains, its mutagenic potency was increased in strains that overexpress nitroreductase (Watanabe *et al.*, 1989; Oda *et al.*, 1992, 1993; Carroll *et al.*, 2002; Østergaard *et al.* 2007). Consolo *et al.* (1989) also confirmed that the mutagenicity of 1-nitropyrene in *Salmonella* was dependent on nitroreductase but not on *O*-esterificase on the basis of results obtained in TA98/1,8DNP_6. Arimochi *et al.* (1998) demonstrated that the mutagenic potency and induction of DNA adducts of 1-nitropyrene were highest in a strain of *Salmonella* that overexpresses nitroreductase (YG1021), were lower in a strain with normal levels of nitroreductase (TA98) and lowest in a strain that had reduced levels of the enzyme (TA98NR).

Further insight into the metabolism of 1-nitropyrene to mutagenic forms in *Salmonella* was obtained by the introduction of derivatives of strain TA98 that overexpress acetyltransferase (YG1024) and overexpress both nitroreductase and acetyltransferase (YG1041) (Hagiwara *et al.*, 1993). The mutagenic potency of 1-nitropyrene relative to that in TA98 was 37.1-fold in YG1021 (overexpressing nitroreductase), 8.2-fold in YG1024 (overexpressing acetyltransferase) and 356.4-fold in YG1041 (overexpressing both enzymes). This study clearly demonstrated the role of *O*-acetyltransferase as well as nitroreductase in the metabolic activation of 1-nitropyrene to a frameshift mutagen. Watanabe *et al.* (1993) noted that the pKM101 plasmid, which is found in strain YG1024 that overexpresses acetyltransferase, was also essential for

1-nitropyrene-enhanced mutagenesis in this strain because the compound was far less mutagenic in an isogenic strain (YG1012) that does not carry the plasmid. Watanabe *et al.* (1990) introduced the *Salmonella* strain YG1029, which is a base-substitution strain derived from TA100 that overexpresses acetyltransferase, that also enhanced the mutagenicity of 1-nitropyrene, which was, however, much less mutagenic at the base-substitution allele than at the frameshift allele.

Some studies have indicated that the metabolic activation of 1-nitropyrene to a mutagen in bacteria may be simpler than and different from that in mammalian systems. Oda *et al.* (1999) showed that 1-nitropyrene was more mutagenic in the *Salmonella umu* test in which human *N*-acetyltransferase 1 was expressed than in a test in which the human *N*-acetyltransferase 2 was expressed, and was least mutagenic in a strain that overexpressed *O*-acetyltransferase. It was also mutagenic in the absence of an exogenous metabolic activation system in the standard *Escherichia coli* PQ37 chromotest for SOS induction (Mersch-Sundermann *et al.*, 1991).

Rosser *et al.* (1996) characterized the mutagenicity of the primary oxidized metabolites of 1-nitropyrene that were identified following mammalian metabolism in strains of *Salmonella* with low, standard or elevated levels of nitroreductase or acetyltransferase. The results indicated that *N*-hydroxylation followed by *O*-esterification, as opposed to further exogenous metabolic activation-catalysed ring-oxidation, was a major route of activation for the urinary metabolites of 1-nitropyrene in rodents.

The roles of other enzymes in the activation of 1-nitropyrene to mutagenic metabolites were investigated in an *umu* assay in *E. coli* into which human CYP1A1, 1A2 and 1B1 had been cloned (Yamazaki *et al.*, 2000); human CYP1B1 was found to activate 1-nitropyrene to a mutagen in this system. Using a strain of *Salmonella* that expressed rat glutathione *S*-transferase 5–5 (NM5004), Oda *et al.* (1996) showed that this enzyme reduced the mutagenic potency of 1-nitropyrene compared with strains in which it was not expressed. The mutagenic potency of 1-nitropyrene was also enhanced by human sulfotransferase 1A1 in a *Salmonella umu* assay expressing this enzyme (Oda *et al.*, 2012).

(ii) Mammalian cell mutagenesis

Since the previous *Monograph* (IARC, 1989), additional studies have confirmed and extended the finding that 1-nitropyrene is mutagenic in mammalian cells *in vivo* and *in vitro*. Germ-free and conventional rats were injected intraperitoneally with radiolabelled 1-nitropyrene and the urinary mutagenicity was evaluated in *S. typhimurium* TA98 in the presence of an exogenous metabolic activation system. The mutagenicity of urine from conventional rats was 10-fold greater than that from germ-free rats, showing that the gut flora was critical to the formation of the mutagenic urinary metabolites (Ball *et al.*, 1991). Conventional rats were administered 1-nitropyrene by gavage or intraperitoneal injection and the urinary mutagenicity was evaluated in *S. typhimurium* TA98 and TA100. Base-substitution urinary mutagenicity (detected by TA100) was produced only by intraperitoneal injection of 1-nitropyrene, whereas frameshift urinary mutagenicity (detected by TA98) was found only after deconjugation and was produced by both routes of exposure (Varga *et al.*, 2006). After intraperitoneal administration of 1-nitropyrene to rats, a considerable amount of the urinary mutagenicity was excreted as glucuronide conjugates (Scheepers *et al.*, 1991). Thus, different types of metabolite are produced depending on the route of exposure. Heflich *et al.* (1990) showed that 1-nitropyrene was mutagenic at the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) gene in Chinese hamster ovary cells only in the presence of exogenous metabolic activation, and that most of the mutagenicity was due to two metabolites – the

4,5- and 9,10-oxides of 1-nitropyrene. The lack of mutagenicity of 1-nitropyrene in the absence of exogenous metabolic activation in the Chinese hamster ovary cell/*Hprt* assay was confirmed by Thornton-Manning *et al.* (1991b), who showed that the compound was also mutagenic in the absence of exogenous metabolic activation at the *Hprt* locus in a nucleotide repair-deficient Chinese hamster ovary cell line (CHO-UV5). Using this repair-deficient cell line, Thornton-Manning *et al.* (1991a) tested the mutagenicity of 1-nitropyrene under aerobic and anaerobic metabolic conditions and analysed the metabolites. The results suggested that the exogenous metabolic activation-mediated mutagenicity under anaerobic conditions produced mutations and DNA adducts that resulted from nitroreductive metabolism, whereas those produced under aerobic conditions resulted from ring-oxidized metabolism.

Kappers *et al.* (2000) demonstrated that 1-nitropyrene was also not mutagenic at the *Hprt* gene in Chinese hamster V79 cells that express either CYP1A2 or CYP3A4 in the absence of exogenous metabolic activation; however, it was mutagenic at a *lacZ* reporter gene in NIH/3T3 cells that express CYP1A2. [The Working Group noted that this result implied the requirement of sulfotransferase for metabolic activation.]

1-Nitropyrene was also mutagenic in human HepG2 cells at the *Hprt* gene in the absence of exogenous metabolic activation, indicating that this cell line has sufficient nitroreductase and other metabolic enzymes to convert the compound to a mutagen (Silvers *et al.*, 1994). Similarly, 1-nitropyrene was mutagenic at the *Hprt* gene in a human B-lymphoblastoid cell line (h1A1vs) that constitutively expresses CYP1A1 in the absence of exogenous metabolic activation (Durant *et al.*, 1996).

(iii) Other systems

1-Nitropyrene induced gene mutation, gene conversion and crossing-over, but not chromosome loss, in the yeast *Saccharomyces cerevisiae* in the absence of exogenous metabolic activation (Rhenimi *et al.*, 2008). When soya bean seeds were exposed to 1-nitropyrene, an increased frequency of yellow and dark green spots on the leaves was found in the adult plant, which indicated the induction of gene mutation; however, no increase in twin spots was observed, indicating that no induction of somatic crossing-over had occurred (Katoh *et al.*, 1994).

(iv) Mutational mechanisms

The mechanisms by which 1-nitropyrene or its metabolites induce gene mutation (base substitutions, small deletions, insertions or frameshifts within a gene) are reviewed below; however, no data were available on the mechanisms by which 1-nitropyrene induces chromosomal mutations (chromosomal aberrations or micronuclei).

In cell-free studies, dG-C8-AP was part of a fragment of DNA or a plasmid to which nucleotide excision-repair enzymes from bacteria or human cell extracts were added. The results showed that the repair complex recognized a large (six-base) bulge in which the adduct was located and that repair was efficient when a C was opposite, but inefficient when no base was opposite, the adducted G. The aminopyrene moiety intercalated, and the adducted G and paired C were displaced into the major groove of the DNA (Mao *et al.*, 1996; Gu *et al.*, 1999; Nolan *et al.*, 1999; Hoare *et al.*, 2000; Sherrer *et al.*, 2009). An in-vitro study showed that the dG-C8-AP lesion is a strong block of DNA replication but, when translesion synthesis occurs, it is largely accurate (Vyas & Basu, 1995).

In bacteria, analysis by probe hybridization and polymerase chain reaction/DNA sequencing of revertants of *Salmonella* exposed to 1-nitropyrene (in the absence of exogenous metabolic activation) showed that 67% of the base

substitutions in TA100 were G→T and 28% were G→A (DeMarini *et al.*, 1996; DeMarini, 2000). The enhanced mutagenic potency of 1-nitropyrene in constructed homologues of *S. typhimurium* TA100 (SN13497, SN15939 and SN15942) compared with that in the parent TA100 was due to the deletion of not only *moeA* and *uvrB* but also that of other genes in TA100 (Swartz *et al.*, 2007). Molecular analysis of 1-nitropyrene-induced revertants of TA98 showed that 100% of the frameshift mutations were a two-base GC deletion within a repeating GC site (Bell *et al.*, 1991; DeMarini *et al.*, 1996; DeMarini, 2000), and that the induction of this mutation required the presence of *umuDCST*, but not *samAB* (Nohmi *et al.*, 1995).

Molybdenum hydrolase is involved in the nitroreduction of 1-nitropyrene; the deletion of both *uvrB* and *moeA* (a gene involved in molybdenum cofactor biosynthesis) from *S. typhimurium* TA100 resulted in an increase in the base-pair mutagenic potency of 1-nitropyrene compared with that in TA98; thus, the absence of other genes that were also deleted in TA98 caused a reduction in the mutagenic potency of 1-nitropyrene compared with that in strains in which no genes other than *uvrB* and *moeA* were deleted (Swartz *et al.*, 2007). 1-Nitropyrene was not mutagenic at either the base-substitution (*hisG46*) or frameshift (*hisD3052*) allele of *Salmonella* when only *moeA* was deleted; the deletion of *uvrB* also was necessary for the mutagenicity of 1-nitropyrene at these alleles (Swartz *et al.*, 2007). Moreover, 1-nitropryene required the pKM101 plasmid, which provides SOS repair, to induce frameshift mutations (TA98), but required both pKM101 and deletion of *uvrB* to induce base-substitution mutations (TA100) in *Salmonella* (DeMarini *et al.*, 1996).

The important influence of the DNA sequence context on the mutation spectra induced was illustrated by the finding that treatment of *Salmonella* strains of the 7001–7006 series (each of which reverts by a specific base substitution) resulted in G→T being the primary mutation followed by G→C (Watanabe *et al.*, 1997). This contrasts with the studies reviewed above, in which the secondary class of base substitution (in TA100) was G→A. Nevertheless, in both *Salmonella* systems, the primary base substitution induced by 1-nitropyrene was G→T. Moreover, 1-nitropyrene was seven times more mutagenic in TA98 (frameshift) than in TA100 (base substitution) (DeMarini, 2000).

In *E. coli*, the nucleotide excision-repair system recognized distortions (a bulge) in the DNA helix when 1-nitropyrene was adducted to double-stranded DNA (Watanabe-Akanuma & Ohta, 1994), as was noted *in vitro* (Zou *et al.*, 2003). However, the nucleotide excision-repair system also recognized the actual chemical modification (i.e. the aminopurine adduct) in single-stranded DNA (Watanabe-Akanuma & Ohta, 1994). As in *Salmonella*, SOS repair in *E. coli* resulted in the induction of –1 or +1 frameshift mutations by 1-nitropyrene, as well as G→T base substitutions; however, C→T base substitutions and two-base GC deletions tended to be induced in the absence of SOS (Stanton *et al.*, 1988; Malia & Basu, 1994; Melchior *et al.*, 1994; Malia & Basu, 1995; Malia *et al.*, 1996; Bacolod *et al.*, 2000; Luo *et al.*, 2000; Bacolod & Basu, 2001; Hilario *et al.*, 2002).

In mammalian cells, 1-nitrosopyrene or dG-C8-AP induced primarily G→T or G→A base substitutions (Yang *et al.*, 1988; McGregor *et al.*, 1994; Watt *et al.*, 2007). A direct comparison of the mutation spectra induced by dG-C8-AP at the underlined G in the sequence CGCGCG resulted in a G→T base substitution when this construct was replicated in mammalian cells (Watt *et al.*, 2007) but in a two-base CG deletion when it was replicated in bacterial cells (Hilario *et al.*, 2002). This comparison illustrated variations in the way in which the same adduct within the same DNA sequence context can be processed by different cells into a different spectrum of mutations.

Similarly, the influence of sequence context on the resulting mutation spectrum was illustrated

in mammalian cells, in which the 9,10-oxide of 1-nitropyrene was two- to threefold more mutagenic than the 4,5-oxide in either the Chinese hamster ovary cell/*Hprt* (Kim *et al.*, 2005b) or at the *SupF* gene in a shuttle vector in human XP-A fibroblasts (Kim *et al.*, 2008). However, these compounds induced primarily G→A, followed by G→T, base substitutions at *Hprt*, whereas they induced primarily G→T base substitutions at *SupF*.

The potential mutation spectrum of 1-nitropyrene is highly dependent on the type, the DNA sequence context (neighbouring DNA sequence), the methylation status of the DNA and the DNA repair status of the cell. Few generalizations emerged from the studies reviewed here; however, base substitutions targeted primarily at G and, to a certain extent, at A, as well as small frameshifts, were the primary classes of mutation induced by the active metabolites of 1-nitropyrene. The frameshifts could generally be explained by a standard slippage model (DeMarini *et al.*, 1998; DeMarini, 2000) and the base substitutions by polymerase misincorporation (Sherrer *et al.*, 2009).

(d) Cytogenetic effects

1-Nitropyrene induced chromosomal aberrations in Chinese hamster Don:Wg3H cells in the absence of exogenous metabolic activation, producing primarily chromatid exchanges and chromosomal and chromatid aberrations (Lafi & Parry, 1987). However, in the Chinese hamster lung cell line, 1-nitropyrene induced chromosomal aberrations in the presence but not in the absence of exogenous metabolic activation (Matsuoka *et al.*, 1991). One study reported the induction of chromosomal aberrations in the bone marrow of mice injected intraperitoneally with 1-nitropyrene (Pusztai *et al.*, 1998).

1-Nitropyrene induced micronuclei in human lymphocytes *in vitro*; the majority (62%) of the micronuclei did not hybridize with a centromeric probe, indicating that they were caused by chromosome breakage as opposed to aneuploidy (Bonnefoy *et al.*, 2012). It also induced micronuclei in mouse liver *in vivo* (Igarashi *et al.*, 2010).

1-Nitropyrene did not induce cellular transformation in rat tracheal epithelial cells *in vitro* (Mitchell & Thomassen, 1990; West & Rowland, 1994; Ensell *et al.*, 1998), but did induce transformation in cells that had been removed from the tracheas of 1-nitropyrene-treated rats and cultured *in vitro* (Ensell *et al.*, 1998). Approximately 50% of the transformed foci produced *in vivo* were able to be passaged > 20 times to become immortal cell lines (Ensell *et al.*, 1998). Furthermore, all five of the 1-nitropyrene-induced cell lines displayed anchorage-independent growth and grew in nude mice, indicating that 1-nitropyrene-transformed cells had a high probability of developing into tumours (Ensell *et al.*, 1999). Sheu *et al.* (1994) found that 1-nitropyrene induced a significant increase in the frequency of BALB/3T3 A31-1-1 cell transformation *in vitro* at the highest level tested.

(e) Oncogenes and tumour-suppressor genes

A/J mice received an intraperitoneal injection of 1-nitropyrene and were examined for lung tumours 24 weeks later. K_i-*Ras* mutations were found in 75% of adenocarcinomas, 26% of adenomas and 12% of hyperplasias; no mutations were found in normal tissues adjacent to the tumour (Bai *et al.*, 1998). The most frequent *Ki-ras* mutation was the arginine (CGA) AT→GC transition at codon 61 in exon 2. In addition, the frequencies of tumours that expressed proliferating-cell nuclear antigen and silver-staining nucleolar organizer regions were higher among those with K_i-*Ras* mutations than in those with no such mutations.

CAA→CGA mutations in codon 61 or GGT→GAT mutations in codon 12 of the K_i-*Ras* gene were found in the combined lung adenomas and adenocarcinomas from A/J mice 18 weeks after intraperitoneal administration of 1-nitropyrene (Nakanishi *et al.*, 2001).

Intraperitoneal administration of 1-nitropyrene to CBA/Ca mice followed by examination 48 hours later revealed elevated expression of Ha-*Ras* in the liver, lung, kidney, spleen and thymus, with the largest increase in the lung; the increases were greater in males than in females in all organs (Pusztai *et al.*, 1998). 1-Nitropyrene has been shown to increase the expression of c-*Myc* in the spleen, lymph and bone marrow, but to decrease its expression in the thymus of CBA/Ca mice (Ember *et al.*, 2000). The authors also found showed that 1-nitropyrene increased the expression of *p53* in the spleen and bone marrow but not in the lymph of these mice. In rats, 1-nitropyrene increased the expression of Ha-*Ras* and *p53* in leukocytes and the spleen but not in the lung, liver or kidney (Ember *et al.*, 2000).

(f) Gene expression

1-Nitropyrene induced apoptosis in mouse Hepa1c1c7 cells (Landvik *et al.*, 2007) via a caspase- and AMP-activated protein kinase-dependent pathway involving CYP1A1 that is also associated with a decrease in the expression of stearoyl-coenzyme A desaturase 1, resulting in an alteration in lipid homeostasis (Podechard *et al.*, 2011). In this regard, 1-nitropyrene has also been shown to stabilize the mRNA of CYP1A1 via the Akt pathway (Chu *et al.*, 2009), and the expression of p53 protein that it induced was mediated by CYP1A1 (Su *et al.*, 2011). The induction of apoptosis in mouse Hepa1c1c7 cells by 1-nitropyrene also involved ionic imbalance, as indicated by an intracellular accumulation of Ca^{2+}, as well as oxidative damage (Asare *et al.*, 2009). 1-Nitropyrene also increased the ubiquitination of p21 protein after the stabilization of p53 and expression of p21 (Nakanishi *et al.*, 2000). A study in human HepG2 cells found that aldo-keto reductase 1C2 was essential for the induction of p53 phosphorylation and apoptosis by 1-nitropyrene (Su *et al.*, 2008).

1-Nitropyrene induced reactive oxygen species and the expression of the endoplasmic reticulum stress chaperone protein GRP78 in human umbilical vein endothelial cells *in vitro* (Andersson *et al.*, 2009). It induced interleukin (IL)-8, IL-6, tumour necrosis factor-α and CXC chemokine ligand 8 in BEAS-2B cells (Øvrevik *et al.*, 2009, 2010, 2011), as well as inflammation-related genes such as pentaxin, IL-1β, IL-6, IL-8, 2(CXC chemokine ligand 2) and tumour necrosis factor-α in human bronchial epithelial BEAS-2B cells (Park & Park, 2009). 1-Nitropyrene also induced CYP1 mRNA and protein in various human cell lines (Iwanari *et al.*, 2002; Cherng *et al.*, 2006; Hirano *et al.*, 2011).

4.3 Other data relevant

Administration of 1-nitropyrene by gavage to Fischer 344 rats produced γ-glutamyl transpeptidase-positive foci in the liver after partial hepatectomy; such foci are thought to be an early lesion of hepatocarcinogenesis (Denda *et al.*, 1989).

4.4 Mechanistic considerations

The carcinogenicity of 1-nitropyrene in rodents has been reviewed previously (IARC, 1989). Those and more recent studies have demonstrated that 1-nitropyrene was generally carcinogenic in newborn rodents but not in adults, and was not carcinogenic in the following studies in young or adult rodents: by injection in male Fischer 344 rats (Ohgaki *et al.*, 1985), topical application in Crl/Cd-1(ICR) BR mice aged 4–5 weeks (el-Bayoumy *et al.*, 1982), topical application or intraperitoneal injection in SENCAR mice aged 7 weeks (Nesnow *et al.*, 1984), subcutaneous injection in BALB/c mice (Tokiwa *et al.*, 1984), intrapulmonary implantation in Fischer 344/DuCrj rats (Maeda *et al.*, 1986) or gavage in CD rats (el-Bayoumy *et al.*, 1995). In contrast, it was carcinogenic in the following studies in newborns: by suprascapular

injection in Sprague-Dawley rats, producing histocytomas at the injection site (Hirose *et al.*, 1984); gavage in Sprague-Dawley rats, producing mammary adenocarcinomas in females (el-Bayoumy *et al.*, 1988); intraperitoneal and subcutaneous injection in weanling CD rats, producing mammary fibroadenomas in females (Imaida *et al.*, 1991b); intraperitoneal injection in weanling CD rats, producing mammary tumours in females (Imaida *et al.*, 1991a); intraperitoneal and subcutaneous injection in B6C3F$_1$ mice, producing liver carcinomas (Wislocki *et al.*, 1986); and intraperitoneal injection in female CD and Fischer 344 rats, producing mammary tumours in the CD rats and leukaemia in the Fischer 344 rats (Imaida *et al.*, 1995). However, intraperitoneal injection of newborn B6C3F$_1$ mice with either the 4,-5-oxide or 9,10-oxide of 1-nitropyrene was not carcinogenic (Mori *et al.*, 1992).

An explanation for the carcinogenicity of 1-nitropyrene in newborns but not in adults has been proposed by Silvers *et al.* (1997), who noted that newborn rodents have a low ratio of ring-oxidation to nitroreduction compared with adults, and therefore also have a relatively low ratio of CYP levels to nitroreductase compared with adults. Thus, newborns would favour the nitroreductase (activation) pathway more than the inactivation (oxidation) pathway, whereas the converse would occur in adults. Consequently, newborns are more liable than adults to reduce 1-nitropyrene to hydroxylamine and to the mutagenic dG-C8-AP adduct, which could lead to cancer. Conversely, adult rodents would be more liable to oxidize 1-nitropyrene to phenols, which do not form adducts, are not mutagenic and would not cause cancer.

Silvers *et al.* (1997) also considered the comparison between rodents and humans, and noted that human liver microsomes converted 1-nitropyrene by CYP3A4 to 1-nitropyrene-3-ol, whereas other CYPs were involved in rodents, the liver microsomes of which produced only the 6-ol and 8-ol of 1-nitropyrene. They also noted that the ratio of ring-oxidation to nitroreduction was sufficiently low in human HepG2 liver cells for 1-nitropyrene to be metabolized preferentially via nitroreductase (an activation pathway) in human liver, resulting in the formation of the dG-C8-AP adduct and mutation in human HepG2 cells *in vitro*. Thus, adult rodents may not be a suitable model for the human metabolism of 1-nitropyrene, which may favour activation via nitroreductase rather than inactivation via CYP pathways.

An inverse association between 1-nitropyrene in the lung and 5-year survival from lung cancer in humans has been identified (Tokiwa *et al.*, 1998). The concentration of 1-nitropyrene was measured in 256 human lung specimens with carcinoma and did not differ significantly from that in a set of lung samples from patients with tuberculosis. However, the 5-year survival was markedly lower among lung cancer patients with 1-nitropyrene concentrations > 18 pg/g of lung tissue compared with those who had concentrations < 18 pg/g.

5. Summary of Data Reported

5.1 Exposure data

1-Nitropyrene is an incomplete combustion product and does not appear to be formed in the atmosphere. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. In exhaust emissions and in ambient air, 1-nitropyrene is associated with particles and is not observed in the gas phase. The most important source of 1-nitropyrene is from traffic emissions. In diesel and gasoline engine exhaust emissions, 1-nitropyrene is the most abundant nitroarene. It has also been found in emissions from industrial sources, such as waste incinerators and coke ovens. Other measurements of 1-nitropyrene

in the outdoor environment (surface water, water sediments, snow and rain) can probably be explained by the aforementioned primary combustion sources. During sampling for 1-nitropyrene from emissions with high concentrations of nitrogen oxides, pyrene could possibly be converted to 1-nitropyrene on the medium used. This artificial source of 1-nitropyrene is thought to be negligible when particles are collected from ambient air. Workers who operate, repair or work close to idling or moving diesel-powered vehicles or stationary equipment are probably exposed to 1-nitropyrene at concentrations in the range of 0.1–2.5 ng/m^3. Three overlapping subcategories can be distinguished: occupations with high exposures associated with the use or repair of diesel engines in confined spaces, such as mining and repair shops, with concentrations in the 1.0–2.5 ng/m^3 range; other indoor use of diesel-powered engines, such as indoor use of forklifts, leading to exposures ranging from 0.1 to 1.5 ng/m^3; and outdoor use of diesel engines, in occupations such as a driver, resulting in exposures below 0.5 ng/m^3. Potential sources of exposure for the general population are indoor and outdoor air, and indoor and domestic use of liquefied petroleum gas, kerosene heaters and oil fumes from cooking. The presence of 1-nitropyrene as a food contaminant can probably be explained by its ubiquitous occurrence as an air pollutant, although it may be produced by some food processing, such as smoking, grilling and roasting. Foodstuffs with the highest observed levels of contamination include tea, spices and some cooking oils. In addition, dietary intake can originate from the preparation of foods, such as grilled or smoked meat or fish. 1-Nitropyrene has not been detected in tobacco smoke.

In the general environment, 1-nitropyrene is the most frequently used chemical marker to characterize nitroarenes in the particulate matter of ambient air. 1-Nitropyrene has been used to characterize these compounds in many urban, rural and remote locations worldwide. In outdoor air, the highest concentrations were observed in urban areas, and were presumably related to vehicle exhaust emissions. Ambient air concentrations ranged from 10 to 1000 pg/m^3 in urban areas and from 1 to 100 pg/m^3 in rural and remote areas with low traffic intensity. The concentrations of 1-nitropyrene tend to be higher during the cold seasons (winter) than during the warm seasons (summer).

1-Nitropyrene is metabolized to 1-aminopyrene, which can be measured in human tissues, and was detected in the urine of workers exposed to exhaust from operating diesel-powered equipment. 1-Aminopyrene was also found in the blood of health-care workers employed in an urban environment and in the blood of office workers who lived in a rural environment.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1-Nitropyrene was tested for carcinogenicity by subcutaneous injection in one study, by intraperitoneal injection in four studies and in two initiation–promotion studies in mice; by oral administration in three studies, by subcutaneous injection in five studies, by intraperitoneal injection in three studies, by implantation into the lung in one study and by direct injection into the mammary area in one study in rats; and by intratracheal instillation in one study in hamsters.

In mice, subcutaneous injection of 1-nitropyrene did not cause an increase in the incidence of tumours at any site. Intraperitoneal injection caused a significant increase in the incidence of liver tumours in males in one study and of lung tumours in males and females in another study. The other two studies by intraperitoneal injection

gave negative results, as did the two skin tumour initiation–promotion studies.

Oral administration of 1-nitropyrene to rats caused a significant increase in the incidence of mammary tumours (mostly benign) in females in two studies and of lung tumours in males in one study. The third study of oral administration gave negative results. Subcutaneous injection to rats caused a significant increase in the incidence of malignant tumours at the injection site in one study and of mammary tumours in two studies. Two other studies of subcutaneous injection gave negative results and another was inadequate for an evaluation of carcinogenicity. Intraperitoneal injection to rats caused a significant increase in the incidence of mammary tumours in one study but gave negative results in two other studies. Studies of the implantation of 1-nitropyrene into the lung or injection into the mammary area of rats also gave negative results.

In hamsters, intratracheal instillation did not cause a significant increase in the incidence of tumours at any site.

5.4 Mechanistic and other relevant data

1-Nitropyrene is a constituent of diesel exhaust, and its concentration in air has been used as a marker for exposure to diesel exhaust. In addition, urinary metabolites of 1-nitropyrene have been detected in people who worked in diesel-contaminated atmospheres, suggesting that such metabolites may be a suitable marker for exposure to diesel exhaust in humans. 1-Nitropyrene is metabolized by a combination of ring-oxidation and nitroreduction, and the gut flora also plays a role. Bacterial studies have implied that molybdenum hydrolase is also involved in the metabolism and base-substitution mutagenicity of 1-nitropyrene. The compound is metabolized to hydroxyl amino metabolites that are electrophilic and form DNA adducts, the primary form of which is *N*-(deoxyguanosin-8-yl)-1-aminopyrene.

The DNA damage induced by the metabolites of 1-nitropyrene produced mutations, primarily frameshifts in bacterial systems and base substitutions (mostly GC→TA) in mammalian cells. Standard slippage models explained the frameshifts, and polymerase misincorporation models explained the base substitutions. In addition to gene mutation, 1-nitropyrene also caused chromosomal mutation, such as micronucleus formation, and morphological cell transformation. In contrast to the mutation spectrum of 1-nitropyrene in mammalian cells *in vitro* or in bacterial cells, oncogenes in 1-nitropyrene-induced tumours in rodents have primarily AT→GC mutations, as well as altered patterns of expression.

1-Nitropyrene was carcinogenic in newborn but not in adult rodents. This was probably due to the increased ratio of nitroreduction to ring-oxidation in newborn compared with adult animals; the nitroreduction pathway leads to the formation of mutagenic and carcinogenic metabolites, whereas ring-oxidation is a detoxification route. In human liver cells *in vitro*, the ratio of nitroreduction to ring-oxidation was sufficiently high to result in the preferential activation of 1-nitropyrene via nitroreductase. Thus, adult rodents may not be a suitable model for the human metabolism of 1-nitropyrene. Similarly, the formation of urinary metabolites of 1-nitropyrene in humans favours the nitroreduction pathway. 1-Nitropyrene also induced oxidative stress and the formation of reactive oxygen species, inflammatory proteins and apoptosis in mammalian cell systems and rodents. These mechanisms, together with its direct genotoxicity, could contribute to the carcinogenicity of 1-nitropyrene.

Overall, these data provide *strong mechanistic evidence* to support the carcinogenicity of 1-nitropyrene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1-nitropyrene.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

1-Nitropyrene is *probably carcinogenic to humans (Group 2A).*

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