

# 1,6-DINITROPYRENE

1,6-Dinitropyrene 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

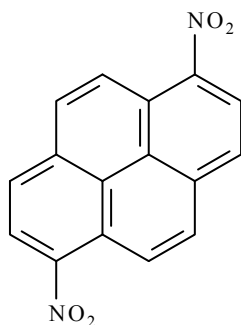
#### 1.1.1 Nomenclature

*Chem. Abstr. Serv. Reg. No.:* 42397-64-8

*Chem. Abstr. Name:* Pyrene, 1,6-dinitro-

*IUPAC Systematic Name:* 1,6-Dinitropyrene

#### 1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

*Relative molecular mass:* 292.3

#### 1.1.3 Chemical and physical properties of the pure substance

*Description:* Light-brown needles, recrystallized from benzene and methanol ([Buckingham, 1985](#))

*Melting-point:* > 300 °C ([Buckingham, 1985](#)); 309–310 °C ([Paputa-Peck et al., 1983](#))

*Spectroscopy data:* Ultraviolet ([Paputa-Peck et al., 1983](#)), infrared ([Hashimoto & Shudo, 1984](#)), nuclear magnetic resonance ([Kaplan, 1981](#); [Paputa-Peck et al., 1983](#); [Hashimoto & Shudo, 1984](#)) and mass spectral data ([Schuetzle, 1983](#)) have been reported. The National Institute of Standards and Technology Chemistry WebBook provides extensive spectroscopic data ([Linstrom & Wallard, 2011](#)).

*Solubility:* Moderately soluble in toluene ([Chemsyn Science Laboratories, 1988](#))

#### 1.1.4 Technical products and impurities

1,6-Dinitropyrene is available for research purposes at a purity of 98% ([Sigma-Aldrich, 2012](#)). The ChemicalBook web site lists 18 companies that supply 1,6-dinitropyrene ([ChemicalBook, 2012](#)).

**Table 1.1 Levels of 1,6-dinitropyrene in diesel engine exhaust particles and their extracts**

Reference	Vehicle/engine	Concentration of 1,6-DNP (pg/mg particulate matter)
<a href="#">Nishioka et al. (1982)</a>	Passenger cars (LDD)	ND–600 <sup>a</sup>
<a href="#">Gibson (1983)</a>	Diesel cars 1978–82 (LDD)	33–34
<a href="#">Nakagawa et al. (1983)</a>	Idling 6-tonne bus from 1970 (HDD), 1200 rpm	1.2
<a href="#">Schuetzle &amp; Perez (1983)</a>	<i>Heavy-duty vehicle</i>	
	Idle	< 800
	High-speed, no load	1200
	High-speed, full load	800
<a href="#">Salmeen et al. (1984)</a>	Passenger cars (LDD)	400 ± 200
<a href="#">Manabe et al. (1985)</a>	Four-cycle six-cylinder engine (HDD) at 17.7–40 km/h	810
<a href="#">Tokiwa et al. (1986)</a>	Idling engine [not further specified]	13
<a href="#">Draper (1986)</a>	Commercial mining engine (HDD), 100% load, 1200 rpm	ND (< 230)
	Commercial mining engine (HDD), 75% load, 1800 rpm	ND (< 1100)
<a href="#">Hayakawa et al. (1992)</a>	Idling engine (LDD)	113.9 <sup>b</sup>

<sup>a</sup> Range of three different engines

<sup>b</sup> Using a much more sensitive analytical method

HDD, heavy-duty diesel; LDD, light-duty diesel; ND, not detected

## 1.2 Analysis

The reader is referred to Section 1.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

## 1.3 Production and use

The reader is referred to Section 1.3 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

## 1.4 Occurrence and environmental exposure

### 1.4.1 Engine exhaust

The reader is referred to the *Monographs* on Diesel and Gasoline Engine Exhausts and 1-Nitropyrene in this Volume.

During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of 1,3-, 1,6- and 1,8-dinitropyrene ([Heeb et al., 2008](#)). A variety of tests of diesel engine emissions were performed in the 1980s, which showed a range of concentrations of 1,6-dinitropyrene in the particulate matter (PM) ([Table 1.1](#)). It was

detected at levels of 0.81 ng/mg ([Manabe et al., 1985](#)) and 1.2 ng/mg ([Nakagawa et al., 1983](#)) in extracts of particles from the exhaust of heavy-duty diesel engines; and at 0.4 ± 0.2 ng/mg extract ([Salmeen et al., 1984](#)), 0.6 ng/mg extract ([Nishioka et al., 1982](#)) and 0.033–0.034 ng/mg particles ([Gibson, 1983](#)) from the exhaust of light-duty diesel engines (reviewed in [Fu & Herreno-Saenz, 1999](#)). The production of dinitropyrene therefore appears to depend on engine size and operating conditions.

[Hayakawa et al. \(1992, 1994\)](#) examined nitro-polycyclic aromatic hydrocarbons (PAHs) in PM emissions from 15 diesel and gasoline engine vehicles. Compared with diesel engine exhaust, those of gasoline engines contained approximately twice as much 1,6-dinitropyrene ([128 pg/mg] versus [67 pg/mg]; [Table 1.2](#)); however, the ratio of concentrations of 1,6-dinitropyrene to 1-nitropyrene was 29% for gasoline and 0.5% for diesel engines, which was assumed to be the result of differences in combustion conditions. Diesel engines produced much more PM, and their total emissions of dinitropyrene isomers were much greater. In air

**Table 1.2 Mass concentrations of particulate matter from diesel and gasoline engine exhausts from tailpipes in 1992**

	No. of samples	Concentration (pg/mg) <sup>a</sup>			
		1-NP	1,3-DNP	1,6-DNP	1,8-DNP
Gasoline engine, idle	8	444 ± 210	64 ± 44	128 ± 106	102 ± 53
Diesel engine, idle	7	12 600 ± 13 100	67 ± 44	67 ± 47	61 ± 41

<sup>a</sup> Values are the means ± standard deviations  
DNP, dinitropyrene; NP, nitropyrene  
From [Hayakawa et al. \(1992, 1994\)](#)

concentrations of emissions from mixed traffic, the ratio of 1,6-dinitropyrene to 1-nitropyrene decreased as the relative number of diesel vehicles increased.

In the past decade, particulate filters have been developed to filter PM from diesel engine exhaust to control emissions. The accumulated soot particles and organic carbon components, including PAHs and nitro-PAHs, that collect on the filters are removed by oxidation, aided by catalytic coatings or catalysts added to the fuel (see Section 1.1 of the *Monograph on Diesel and Gasoline Engine Exhausts* in this Volume).

In a series of laboratory tests, a range of diesel particulate filter types were tested to compare their impact on PAH and nitro-PAH emissions ([Heeb et al., 2010](#)). All filters tested, which removed 99% of the particles, also removed most PAH components. However, low-oxidation filters produced 63% more 1-nitropyrene than the amount present in the unfiltered exhaust; although they were not measured, the quantities of dinitropyrenes would also be expected to increase similarly.

[Carrara & Niessner \(2011\)](#) examined the formation of 1-nitropyrene in high- and low-oxidation filters operating at temperatures of 293–573 °K (20–300 °C). Lower temperatures produced more 1-nitropyrene on the filter, the level of which peaked at ~100 °C and declined at higher temperatures. Measurements at 250 °C showed that < 2% of the 1-nitropyrene was on the filter and 47% ± 12% was on the vapour collector

(losses of vapour were noted). Although they were not measured in the samples, dinitropyrenes would be expected to be similarly affected.

#### 1.4.2 Environmental occurrence in air and water

The nitration of pyrene during atmospheric processes leads to the formation of 2- but not 1-nitropyrene, because the oxidants that are present differ from those that occur during combustion, which produces 1-nitropyrene ([Pitts, 1987](#)). Thus, dinitropyrenes are not produced by atmospheric processes.

The presence of dinitropyrenes [not characterized further] in respirable particles from ambient atmospheric samples was inferred from the mutagenicity testing of extracts of polycyclic organic matter ([Pitts, 1987](#)). Early sampling data collected in several locations showed a wide range of concentrations of 1,6-dinitropyrene ([Table 1.3](#)). In remote, rural or unindustrialized areas, the content of 1,6-dinitropyrene in airborne PM was in the range of 4.6–8.3 pg/mg and the corresponding levels in air were 0.12–0.30 pg/m<sup>3</sup>. In contrast, the PM from the heavily industrialized areas had a content of 43–46 pg/mg, with air concentrations of 4.44–7.50 pg/m<sup>3</sup> ([Gibson, 1986](#)). The large urban cities of Tokyo, Japan, and Santiago, Chile, had levels of 1,6-dinitropyrene ranging up to 200 pg/mg ([Tokiwa et al., 1983](#); [Tanabe et al., 1986](#)). One study in Michigan, USA, found much lower levels of 1,6-dinitropyrene than other investigators ([Siak et al., 1985](#)).

**Table 1.3 Concentrations of 1,6-dinitropyrene in air samples and collected particulate matter**

Reference	Site/country Season	Concentration	
		Particulate matter (pg/mg)	Atmosphere (pg/m <sup>3</sup> )
<a href="#">Tokiwa et al. (1983)</a>	Santiago, Chile (urban)	200	–
<a href="#">Siak et al. (1985)</a>	South-eastern MI, USA Summer	0.26–0.35	0.020–0.032
<a href="#">Gibson (1986)</a>	Bermuda (remote) Summer	8.1	0.15 <sup>a</sup>
	Winter	8.3	0.12 <sup>a</sup>
	Delaware, USA (rural) Summer	4.9	0.12 <sup>a</sup>
	Warren, MI, USA (suburban) Winter	< 6	0.15 <sup>a</sup>
	Summer	4.6	0.30 <sup>a</sup>
	Detroit, MI, USA (urban) Summer	3.6	0.48 <sup>a</sup>
	River Rouge, MI, USA (industrial) Summer	46	4.44 <sup>a</sup>
	Dearborn, MI, USA (industrial) Summer	41	7.50 <sup>a</sup>
<a href="#">Tanabe et al. (1986)</a>	Tokyo, Japan	4.7–105	0.33–8.74

<sup>a</sup> Calculated by the IARC Working Group ([IARC, 1989](#))

More recent studies have assessed 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrene in ambient air simultaneously. These studies are presented in Section 1.4.2 of the *Monograph* on 1,3-Dinitropyrene in this Volume.

### 1.4.3 Other sources

Small amounts of dinitropyrenes are generated by kerosene heaters, which are used extensively in Japan to heat residences and offices ([Tokiwa et al., 1985](#)). Such open, oil-burning space heaters were found to emit dinitropyrenes at a rate of 0.2 ng/h after one hour of operations; a mixture of 1,6- and 1,8-dinitropyrenes was found at  $3.25 \pm 0.63$  mg/kg particulate extract.

Gas and liquefied petroleum gas burners are widely used for home heating and cooking. Levels of 1,6-dinitropyrene of 1.88 mg/kg extract were reported from one gas burner ([Tokiwa](#)

[et al., 1985](#)). Dinitropyrenes may result from the incomplete combustion of fuel in the presence of nitrogen dioxide.

Toners for photocopy machines have been produced commercially since the late 1950s and have been in widespread use since that time. ‘Long-flow’ furnace black was first used in photocopy toners in 1967; its manufacture involved an oxidation process, during which some nitration of pyrene also occurred. A carbon black sample manufactured before 1979 was reported to contain 21 mg/kg 1,6-dinitropyrene ([Sanders, 1981](#)); another ‘long-flow’ furnace carbon black sample was also found to contain this compound ([Ramdahl & Urdal, 1982](#)). Toners produced from a new type of carbon black since 1980 had no detectable levels of mutagenicity, and hence of nitropyrenes ([Rosenkranz et al., 1980](#); [Butler et al., 1983](#)). A sample of carbon black made in 1980 contained 0.13 mg/kg 1,6-dinitropyrene

after optimization of the extraction method ([Giammarise et al., 1982](#)).

## 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 Intraperitoneal administration

Groups of 90 or 100 male and female newborn CD-1 mice received three intraperitoneal injections of 1,6-dinitropyrene (purity, > 99%; total dose, 200 nmol [58.7 µg]) or benzo[*a*]pyrene (purity, > 99%; total dose, 560 nmol [140 µg]) in 10, 20 and 40 µL of dimethyl sulfoxide (DMSO) on days 1, 8 and 15 after birth or DMSO alone. At 25–27 days, when the mice were weaned, 25 males and 29 females in the treated group, 37 males and 37 females in the positive-control group, and 28 males and 31 females in the control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,6-dinitropyrene, 8 out of 25 (32%) male mice developed liver tumours (three adenomas, five carcinomas); this incidence was significantly greater than that in the vehicle controls ( $P < 0.025$ ). No increase in the incidence of lung tumours or malignant lymphomas was observed in males or females compared with DMSO-treated animals ([Wislocki et al., 1986](#)).

#### 3.1.2 Subcutaneous administration

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0 (vehicle control) or 0.1 mg of 1,6-dinitropyrene

(purity, > 99,9%) dissolved in 0.2 mL of DMSO once a week for 20 weeks (total dose, 2 mg). Animals were observed for 60 weeks or, for mice that developed tumours at the site of injection, until moribund. The first tumour in the 1,6-dinitropyrene-treated group was seen on day 112; 45 weeks after the first treatment, 10 out of 20 mice ( $P < 0.002$ ) had developed tumours at the injection site that were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for some subcutaneous and intraperitoneal sarcomas]. No subcutaneous tumour was detected in the vehicle controls ([Tokiwa et al., 1984](#)).

### 3.2 Rat

See [Table 3.2](#).

#### 3.2.1 Oral administration

A group of 36 female weanling Sprague-Dawley rats received intragastric intubations of 0 (vehicle control) or 10 µmol [3 mg]/kg body weight (bw) of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL), three times a week for 4 weeks (average total dose, 16 µmol [4.7 mg]/rat) and were observed for 76–78 weeks. Two rats (6%) treated with 1,6-dinitropyrene and none of the controls developed leukaemia. Mammary adenocarcinomas and fibroadenomas were found in 11 out of 36 (31%) and 10 out of 36 (28%) treated animals, respectively, which was not statistically different from the incidence in controls (5 out of 35 (14%) adenocarcinomas and 9 out of 35 (26%) fibroadenomas). Adrenal and pituitary tumours were also observed in treated animals at an elevated but non-significant level compared with controls ([King, 1988](#); [Imaida et al., 1991](#)). [The Working Group noted the short duration of both treatment and observation periods and the use of a single dose.]

**Table 3.1 Studies of the carcinogenicity of 1,6-dinitropyrene in mice**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Newborn CD1 (M, F) 12 mo <a href="#">Wislocki et al. (1986)</a>	Intraperitoneal administration 0 (control), 200 nmol [58.7 mg] 1,6-DNP or 560 nmol [140 µg] B[a]P in 10, 20 or 40 µL DMSO (total dose), at 1, 8 and 15 days after birth Groups of 90 M, 100 F newborn mice	Liver (adenoma): M–2/28 (7%), 3/25 (12%), 11/37 (30%) F–0/31, 0/29, 0/27 Liver (carcinoma): M–0/73, 5/25 (20%), 7/37 (19%) F–0/65, 0/29, 0/27 Lung (adenoma): M–1/28 (4%), 1/25 (4%), 13/37 (35%) F–0/31, 2/29 (7%), 13/27 (48%) Lung (carcinoma): M–0/28 (3%), 0/25, 0/37 F–0/31, 0/29, 0/27 Malignant lymphoma: M–1/28 (4%), 0/25, 2/37 (5%) F–1/31 (1%), 2/29 (15%), 4/27 (15%)	$P < 0.05$ (liver carcinoma in M versus control)	Purity, > 99% Study limited by a small number of animals per group and short observation period.
BALB/c (M) 60 wks or until moribund <a href="#">Tokiwa et al. (1984)</a>	Subcutaneous injection 0 (control) or 0.1 mg 1,6-DNP in 0.2 mL DMSO (total dose, 2.0 mg), once/ wk for 20 wks Groups of 20 aged 6 wks	Site of injection (malignant fibrous histiocytoma): 0/20, 10/20 (50%)	$P < 0.002$	Purity, > 99%

B[a]P, benzo[a]pyrene; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female, M, male; mo, month; wk, week

**Table 3.2 Studies of the carcinogenicity of 1,6-dinitropyrene (1,6-DNP) in rats**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 76–78 wks <a href="#">King (1988)</a> ; <a href="#">Imaida et al. (1991)</a>	Oral administration (intragastric intubation) 0 (control) or 10 µmol [3 mg]/kg bw 1,6-DNP in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 × /wk for 4 wks Groups of 35 or 36 weanlings	Leukaemia: 0/36, 2/36 (6%) Mammary (adenocarcinoma): 5/35 (14%), 11/36 (31%) Mammary (fibroadenoma): 9/35 (26%), 10/36 (28%) Adrenal (pheochromocytoma): 4/36 (11%), 7/36 (19%) Adrenal (cortical adenoma): 6/36 (17%), 7/36 (19%) Pituitary (carcinoma): 2/36 (6%), 12/36 (33%) Pituitary (adenoma): 9/36 (25%), 13/36 (36%)	NS	Purity > 99% Study limited by the short duration of both treatment and observation periods and the use of a single dose
CD (F) 76–78 wks <a href="#">King (1988)</a> ; <a href="#">Imaida et al. (1991)</a>	Intraperitoneal administration 0 (control) or 10 µmol [3 mg] 1,6-DNP/kg bw in DMSO (total dose, 16 µmol [4.7 mg]/rat), 3 × /wk for 4 wks Groups of 36 weanlings	Peritoneal cavity (malignant fibrous histiocytoma): 0/31, 23/23 (100%)	$P < 0.0001$	Purity, > 90% Some early deaths
F344/DuCrj (M) 72 wks <a href="#">Maeda et al. (1986)</a>	Implantation into the lung 0 (control), 0.15 mg 1,6-DNP or 0.5 mg 3-methylcholanthrene in 0.05 mL beeswax:tricaprylin, single injection Groups of 19–31, aged 10–11 wks	Lung (squamous cell carcinoma): 0/31, 21/28 (75%) 19/19 (100%) Lung (undifferentiated carcinoma): 0/31, 0/19, 2/28 (7%)	$P < 0.005$ NS	Purity, > 99.9% Single-dose study
F344 (M) 104 wks <a href="#">Iwagawa et al. 1989</a> ,	Implantation into the lung 0 (control), 0.003, 0.01, 0.03, 0.1 or 0.15 mg 1,6-DNP or 0.03, 0.1, 0.3 or 1.0 mg B[a]P in beeswax Groups: 40, control; 39, 0.003 mg 1,6-DNP; 30, 0.011 mg 6-DNP; 31, 0.03 mg 1,6-DNP; 26, 0.1 mg 1,6-DNP; 9, 0.15 mg 1,6-DNP; 29, 0.03 mg B[a]P; 30, 0.1 mg B[a]P; 29, 0.3 mg B[a]P; 13, 1.0 mg B[a]P, aged 11 wks	Lung (all tumours): 0–0/40 0.003 1,6-DNP–0/39 0.01 1,6-DNP–4/30 (13%) 0.03 1,6-DNP–13/31 (42%) 0.1 1,6-DNP–22/26 (85%) 0.15 1,6-DNP–6/9 (67%) 0.03 B[a]P–1/29 (3%) 0.1 B[a]P–7/30 (23%) 0.3 B[a]P–22/29 (76%) 1.0 B[a]P–9/13 (69%)	$[P < 0.0299]$ $[P < 0.0001]$ $[P < 0.0001]$ $[P < 0.0001]$ NS $[P < 0.0017]$ $[P < 0.0001]$ $[P < 0.0001]$	Purity, > 99.8%

**Table 3.2 (continued)**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) 320 d for treated, 650 d for controls <a href="#">Ohgaki et al. (1985)</a>	Subcutaneous injection 0 (control) or 0.2 mg 1,6-DNP in 0.2 mL DMSO (total dose, 4 mg), twice/wk for 10 wks Groups of 10 or 20, aged 6 wks	Site of injection (subcutaneous sarcoma): 0/20, 10/10 (100%)	$P < 0.0001$	Purity not reported Study limited by the small number of treated animals and the use of only one dose
CD (F) 149 d for treated, 495 d (control group) <a href="#">King (1988)</a> ; <a href="#">Imaida et al. (1991)</a>	Subcutaneous injection Suprascapular injection starting within 24 h of birth; 1st dose: 2.5 $\mu\text{mol}$ 1,6-DNP/kg bw; 2nd and 3rd doses: 5 $\mu\text{mol}$ /kg bw; 4th–8th doses: 10 $\mu\text{mol}$ /kg bw (total dose in DMSO, 6.3 $\mu\text{mol}$ [1.9 mg]); once/ wk for 8 wks Treated groups of 46 newborns; vehicle-control group of 40 newborns	Site of injection (malignant fibrous histiocytoma): 0/40, 46/46 (100%) Leukaemia: 0/40, 9/46 (20%) Mammary (adenocarcinoma): 1/40 (3%), 3/46 (7%) Mammary (fibroadenoma): 6/40 (15%), 0/46 Mammary (adenoma): 1/40 (3%), 2/46 (4%)	$P < 0.0001$      $P < 0.005$	Purity, > 99%

B[a]P, benzo[a]pyrene; bw, body weight; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female; h, hour; M, male; NS, not significant; wk, week



### 3.2.2 Intraperitoneal administration

A group of 36 female weanling CD rats received intraperitoneal injections of 0 (vehicle control) or 10  $\mu\text{mol}$  [3 mg]/kg bw of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7  $\mu\text{mol}$  [0.5 mg]/mL) three times a week for 4 weeks (average total dose, 16  $\mu\text{mol}$  [4.7 mg]/rat) and were then maintained for 76–78 weeks. Treatment with 1,6-dinitropyrene resulted in some early deaths 12–15 weeks after the initial treatment. Tumours were first identified in a rat autopsied 17 weeks after the first injection. All 23 1,6-dinitropyrene-treated animals (100%) that survived longer than 21 weeks had developed malignant fibrous histiocytomas in the peritoneal cavity, whereas none of the vehicle controls that were observed for 76–78 weeks developed these tumours ( $P < 0.0001$ ). Mammary tumours developed in both groups with approximately the same incidence (King, 1988; Imaida *et al.*, 1991). [The Working Group noted the short duration of both treatment and observation periods.]

### 3.2.3 Intrapulmonary administration

A group of 28 male Fischer 344/DuCrj rats, aged 10–11 weeks, received a single injection of 0.05 mL of beeswax:tricaprylin containing 0.15 mg of 1,6-dinitropyrene (purity, > 99.9%) directly into the lower third of the left lung after left lateral thoracotomy. One group of 19 males received a single injection of 0.05 mL of beeswax:tricaprylin containing 0.5 mg of 3-methylcholanthrene [purity unspecified], and another group of 31 males received an injection of beeswax:tricaprylin alone. Animals were observed for 72 weeks after treatment, at which time the experiment was terminated. In the 1,6-dinitropyrene-treated rats, 21 out of 28 (75%) developed squamous cell carcinomas ( $P < 0.005$ ) and 2 out of 28 developed undifferentiated carcinomas of the lung. Squamous cell carcinomas were induced earlier in all 19

rats treated with 3-methylcholanthrene than in those treated with 1,6-dinitropyrene. No squamous cell carcinoma was observed in the control group. Distant metastases of induced tumours were observed in four 1,6-dinitropyrene-treated and one 3-methylcholanthrene-treated rats. The incidence of Leydig-cell tumours of the testis was significantly lower in 1,6-dinitropyrene- and 3-methylcholanthrene-treated rats than in the controls ( $P < 0.005$ ). The incidence of other tumours did not differ among the groups (Maeda *et al.*, 1986).

Groups of male Fischer 344/NSIc rats, aged 11 weeks, received injections of 0 (vehicle control), 0.003, 0.01, 0.03, 0.1 or 0.15 mg of 1,6-dinitropyrene (purity, > 99.8%), or 0.03, 0.1, 0.3 or 1.0 mg of benzo[*a*]pyrene as suspensions in beeswax:tricaprylin (1:1) into the lung after anaesthesia with ketamine hydrochloride. The animals were then observed for up to 104 weeks. The incidence of lung cancers (mainly undifferentiated neoplasms) was 0 out of 39 (0%), 4 out of 30 (13%), 13 out of 31 (42%), 22 out of 26 (85%) and 6 out of 9 (67%) rats in the groups treated with 0.003, 0.01, 0.03, 0.1 and 0.15 mg of 1,6-dinitropyrene, respectively. Benzo[*a*]pyrene induced lung cancers (well differentiated squamous cell carcinomas) in 1 out of 29 (3%), 7 out of 30 (23%), 22 out of 29 (76%) and 9 out of 13 (69%) rats treated with 0.03, 0.1, 0.3 and 1.0 mg, respectively. No lung cancer was found in control rats. Thus, the incidence of lung cancer induced by 1,6-dinitropyrene and benzo[*a*]pyrene showed significant dose dependence. At equal doses, the incidence of lung cancer was much higher with 1,6-dinitropyrene than with benzo[*a*]pyrene, and was still higher with a dose equivalent to one-third of the dose of benzo[*a*]pyrene (Iwagawa *et al.*, 1989).

### 3.2.4 Subcutaneous administration

Ten male Fischer 344/DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.2 mg of 1,6-dinitropyrene ([purity unspecified];

**Table 3.3 Studies of the carcinogenicity of 1,6-dinitropyrene in hamsters**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (M, F); 11 mo <a href="#">Takayama et al. (1985)</a>	Intratracheal instillation 0 (control) or 0.5 mg in 0.2 ml saline, once/wk for 26 wks Groups of 10 M, 10 F aged 10 wks	<i>Lung (adenocarcinoma):</i> M–0/10 10/10* (100%) F–0/10, 9/10* (90%) <i>Myeloid leukaemia:</i> M–0/10, 6/10 (60%)* F–0/10, 6/10* (60%)	*[ $P < 0.05$ ]	Purity > 99.9% Study limited by the small number of animals, short duration of both treatment and observation periods and use of a single dose

F, female, M, male; mo, month; wk, week.

impurities: < 0.05% each of 1,3-dinitropyrene, 1,8-dinitropyrene, 1,3,6-trinitropyrene and 1,3,6,8-tetranitropyrene) dissolved in 0.2 mL of DMSO twice a week for 10 weeks (total dose, 4 mg). A control group of 20 rats received injections of 0.2 mL of DMSO alone. Treated animals were killed on day 320 and control rats on day 650. Sarcomas developed at the site of injection in all dinitropyrene-treated rats between days 103 and 123. No tumour developed at the injection site among control animals ([Ohgaki et al., 1985](#)). [The Working Group noted that, while it recognized the contamination of the study material by other nitropyrenes, the tumour response was so strong that it can be attributed to the exposure to 1,6-dinitropyrene.]

In a lifetime study, a group of 46 female newborn Sprague-Dawley rats received subcutaneous injections of 1,6-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7  $\mu\text{mol}$  [0.5 mg]/mL) into the suprascapular region once a week for 8 weeks (total dose, 6.3  $\mu\text{mol}$  [1.8 mg]). A group of 40 animals injected with DMSO alone served as controls. The average survival time was 149 days for treated rats and 495 days for controls. Malignant fibrous histiocytomas developed rapidly at the site of injection among treated rats; the first tumour was seen 15 weeks after the initial treatment, and by 18 weeks all rats had developed this tumour ( $P < 0.0001$ ). In addition, nine rats had leukaemia ( $P < 0.005$ ). Vehicle controls developed no such malignancies.

Mammary tumours (mainly adenocarcinomas) were observed in 5 out of 46 (11%) treated rats, and 8 out of 40 (20%) controls had mammary tumours (mainly fibroadenomas) ([King, 1988](#); [Imaida et al., 1991](#)).

### 3.3 Hamster

See [Table 3.3](#).

#### 3.3.1 Intratracheal administration

Groups of 10 male and 10 female Syrian hamsters, aged 10 weeks, received intratracheal instillations of 0 (control) or 0.5 mg of 1,6-dinitropyrene (purity, > 99.9%) suspended in 0.2 mL of saline once a week for 26 weeks (total dose, 13 mg). The experiment was terminated at 11 months. Lung adenocarcinomas developed in 10 out of 10 (100%) males and 9 out of 10 (90%) females treated with 1,6-nitropyrene during weeks 20–48; 65% had multiple tumours. In addition, myeloid leukaemia developed in 6 out of 10 (60%) males and 6 out of 10 (60%) females. No tumours were detected in the controls ([Takayama et al., 1985](#)).

## 4. Mechanistic and Other Relevant Data

### 4.1 Absorption, distribution, metabolism and excretion

#### 4.1.1 Humans

No data were available to the Working Group.

#### 4.1.2 Experimental systems

The metabolic activation of 1,6-dinitropyrene has been reviewed previously ([IARC, 1989](#)) and is caused by the reduction of one of the nitro groups to yield the corresponding hydroxylamine derivative, which in turn can undergo acid-catalysed DNA binding or be converted into a highly reactive *O*-acetylated metabolite by bacteria and mammalian acetyltransferases ([Beland & Marques, 1994](#)). The nitroreduction is catalysed by intestinal bacteria, and this metabolic activation pathway has been shown to be responsible for the mutagenicity of 1,6-dinitropyrene in *Salmonella* ([Fu, 1990](#)). In mammalian systems, the nitroreduction is catalysed by a variety of enzymes, including cytosolic aldehyde oxidase, xanthine/xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) quinone oxidoreductase and microsomal NADPH cytochrome P450 reductase ([Bauer & Howard, 1990, 1991](#)). These enzymes are found in a variety of tissues: xanthine oxidase occurs in the liver, intestinal mucosa and mammary glands, and in the milk and colostrum of most mammals ([Howard et al., 1995](#)).

## 4.2 Genetic and related effects

### 4.2.1 Humans

No data were available to the Working Group.

### 4.2.2 Experimental systems

Within 24 hours after the administration of a single intraperitoneal dose of 1,6-dinitropyrene ([33.6  $\mu\text{mol}$ ]) to pre-weanling male CD mice, one major adduct, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene, was identified in the lung and liver; this adduct was also detected in the mammary epithelium, liver, lung, kidney and urinary bladder of rats ([IARC, 1989](#); [Smith et al., 1993](#)).

Oral administration of 1,6-dinitropyrene to rats resulted in the formation of measurable DNA adducts in the intestinal mucosa and urinary bladder; after intraperitoneal injection, higher levels of DNA adducts were found, mostly in the bladder, white blood cells and lung, but only a lower level was found in the liver ([Wolff et al., 1993](#)). Following direct pulmonary instillation of 1,6-dinitropyrene into male Fischer 344 rats, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene was identified in the target (lung) and surrogate tissues (liver, white blood cells and splenic lymphocytes). The extent of the DNA binding by 1,6-dinitropyrene *in vivo* depends on its nitroreduction and *O*-acetylation ([Beland et al., 1985](#)).

The effect of co-administration of 1-nitropyrene on the levels of DNA adducts derived from 1,6-dinitropyrene was investigated ([IPCS, 2003](#)). Following intraperitoneal administration of 100 nmol of 1,6-dinitropyrene to B6C3F1 mice,  $0.46 \pm 0.05$  fmol of DNA adducts/ $\mu\text{g}$  DNA were detected. Co-administration of a 25-fold molar excess of 1-nitropyrene (but not a 2.5-fold excess) increased the level of DNA adducts (derived from 1,6-dinitropyrene) to  $0.59 \pm 0.07$  fmol/ $\mu\text{g}$  DNA. In contrast, co-administration of 25-fold molar excess of pyrene resulted in a significant decrease in 1,6-dinitropyrene–DNA adducts to  $0.34 \pm 0.04$  fmol/ $\mu\text{g}$  DNA. Collectively, these results suggest that the metabolic activation of 1,6-dinitropyrene can be greatly altered by other agents (e.g. other nitro-PAHs or PAHs) found in

complex mixtures, such as diesel exhaust and air pollutants (IPCS, 2003).

In *Salmonella typhimurium*, 1,6-dinitropyrene (0.005 µg/mL) induced DNA damage (IARC, 1989). Its genotoxicity in bacterial systems other than the *Salmonella* microsome system have been reported, and the results, with a few exceptions, are consistent with those in *Salmonella* (IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992; Oda *et al.*, 1992, 1993; Busby *et al.*, 1994; Shimada *et al.*, 1994; Shane & Winston, 1997; Yamazaki *et al.*, 2000). Several mammalian systems were used to demonstrate the genotoxicity of 1,6-dinitropyrene reported previously (IARC, 1989). 1,6-Dinitropyrene induced DNA damage and mutations in several strains of bacteria, and unscheduled DNA synthesis in mouse, rat and human hepatocytes, in rat and human cultured tracheal or bronchial epithelial cells and in rabbit lung cells. It was mutagenic in several mammalian cell lines, induced sister chromatid exchange and chromosomal aberrations in mammalian cells in culture and caused chromatid-type chromosomal aberrations in cultured rat and hamster liver epithelial cells and human fibroblasts. In rats, 1,6-dinitropyrene produced fibrosarcomas that contained activated *H-ras* and *N-ras* oncogenes (IARC, 1989).

A dose-dependent increase in DNA adducts was found in splenic lymphocytes, but not in the lung, following the direct implantation of 1,6-dinitropyrene into the lung of Fischer 344 rats. In parallel, a significant increase of gene mutation frequency (at the hypoxanthine-guanine phosphoribosyltransferase locus) was detected in splenic T lymphocytes (Smith *et al.*, 1993). Beland and his team (IARC, 1989; Beland & Marques, 1994; Beland, 1995) compared adducts in the lung, liver and lymphocytes and gene mutations in splenic T lymphocytes. The outcome of these studies indicated that the levels of 1,6-dinitropyrene that produce lung tumours in a dose-dependent fashion also induced DNA adducts and mutations in T lymphocytes dose-dependently;

however, the dose-response curves for DNA binding differ from those of mutations. Taken together, these authors suggested that mutation in T lymphocytes may be a more sensitive and longer-lasting biomarker than DNA adducts to assess previous exposure to nitro-PAHs.

Gene mutations were analysed in 20 rat lung tumours induced by 1,6-dinitropyrene; five mutations were detected in *k-Ras* codon 12 (four GGT to TGT transversions and one GGT to GAT transition) but not in *k-Ras* codons 13 or 61. Mutations in *p53* exons 5–8 (eight substitutions at G:C base-pairs and one deletion) were identified in 9 out of 20 tumour samples (Smith *et al.*, 1997).

### 4.3 Other relevant data

Studies of the carcinogenicity of 1,6-dinitropyrene in various species were reported previously (IARC, 1989) and are briefly summarized below.

No data were available to the Working Group on the acute toxicity of 1,6-dinitropyrene. Administration of 10 µmol [3 mg]/kg bw of 1,6-dinitropyrene to rats by gavage, three times a week for 4 weeks had no effects on body weight or survival (Imaida *et al.*, 1991). A single injection of 0.15 mg directly into the lower third of the left lung after left lateral thoracotomy in a group of 28 male Fischer 344 rats resulted in the formation of squamous metaplasia of the lung in two rats and granulomatous lesions containing foreign-body giant cells in three rats. Intraperitoneal administration of 1,6-dinitropyrene to young male Sprague-Dawley rats (three times at 2.5 mg/kg bw) resulted in a 2.5-fold increase in the activity of 1-nitropyrene reductase, a carcinogen metabolizing enzyme, in the liver microsomes compared with controls (IARC, 1989).

### 4.4 Mechanistic considerations

See the *Monograph* on 1,8-Dinitropyrene.

## 5. Summary of Data Reported

### 5.1 Exposure data

1,6-Dinitropyrene is produced by the nitration of 1-nitropyrene. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. During the combustion of diesel and gasoline engines, pyrene is nitrated to form 1-nitropyrene, which is further nitrated to form small amounts of dinitropyrenes. This leads to a content of 1,6-dinitropyrene in the range of 0.1–10% relative to the 1-nitropyrene content in diesel and gasoline exhaust particles and of ~1% in airborne particulate matter. 1,6-Dinitropyrene was present at a range of 1–10 ng/g in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly declined from values in the 0.1–10 pg/m<sup>3</sup> range at urban locations to values in the 0.01–0.1 pg/m<sup>3</sup> range at suburban and rural locations.

1,6-Dinitropyrene is also generated by kerosene heaters. No data on occupational exposure were available to the Working Group.

### 5.2 Human carcinogenicity data

No data were available to the Working Group.

### 5.3 Animal carcinogenicity data

1,6-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and one study by subcutaneous injection, in rats in one study by oral administration, one study by intraperitoneal injection, two studies by implantation into the lung and two studies by subcutaneous injection, and in one study in hamsters by intratracheal instillation. Intraperitoneal injection of 1,6-dinitropyrene into newborn mice caused a significant increase in the incidence of liver carcinomas in males and

its subcutaneous injection caused a significant increase in the incidence of malignant subcutaneous histiocytomas in males. In rats, oral intubation with 1,6-dinitropyrene caused a significant increase in the incidence of pituitary carcinomas in females; its intraperitoneal injection caused a significant increase in the incidence of malignant histiocytomas of the peritoneal cavity in females; its implantation into the lung (two studies) caused a significant increase in the incidence of squamous cell carcinomas of the lung in males; and its subcutaneous injection caused injection site sarcomas in males in one study and malignant histiocytomas and leukaemia in females in another study. In hamsters, intratracheal instillation of 1,6-dinitropyrene caused a significant increase in the incidence of lung adenocarcinomas and myeloid leukaemia in males and females.

### 5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism and excretion or genetic and related effects of 1,6-dinitropyrene in humans. Activation of this compound in bacteria or in mammalian cells occurs via the reduction of one nitro group initially to form a nitroso intermediate, that undergoes further reduction to the *N*-hydroxylamino derivative. 1,6-Dinitropyrene was strongly mutagenic in bacteria. It induced DNA-adduct formation and caused mutation in the splenic T lymphocytes of rats, and induced chromosomal aberrations in human fibroblasts. The mutagenicity of 1,6-dinitropyrene is related to the ability of its corresponding hydroxylamino derivative to bind to DNA. *O*-Acetylation of the *N*-hydroxylamino group by acetyltransferases followed by removal of the acetoxy group yields the active electrophilic nitrenium ion, which reacts with deoxyguanosine at the C8 position

to form *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene. Mutations in the *K-Ras* oncogene and the *Tp53* tumour-suppressor gene were observed in 1,6-dinitropyrene-induced lung tumours in rats. 1,6-Dinitropyrene-induced rat fibrosarcomas contained activated *H-ras* and *N-ras* oncogenes.

Overall, these data provide *moderate mechanistic evidence* to support the carcinogenicity of 1,6-dinitropyrene.

## 6. Evaluation

### 6.1 Cancer in humans

No data were available to the Working Group.

### 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,6-dinitropyrene.

### 6.3 Overall evaluation

1,6-Dinitropyrene is *possibly carcinogenic to humans* (Group 2B).

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