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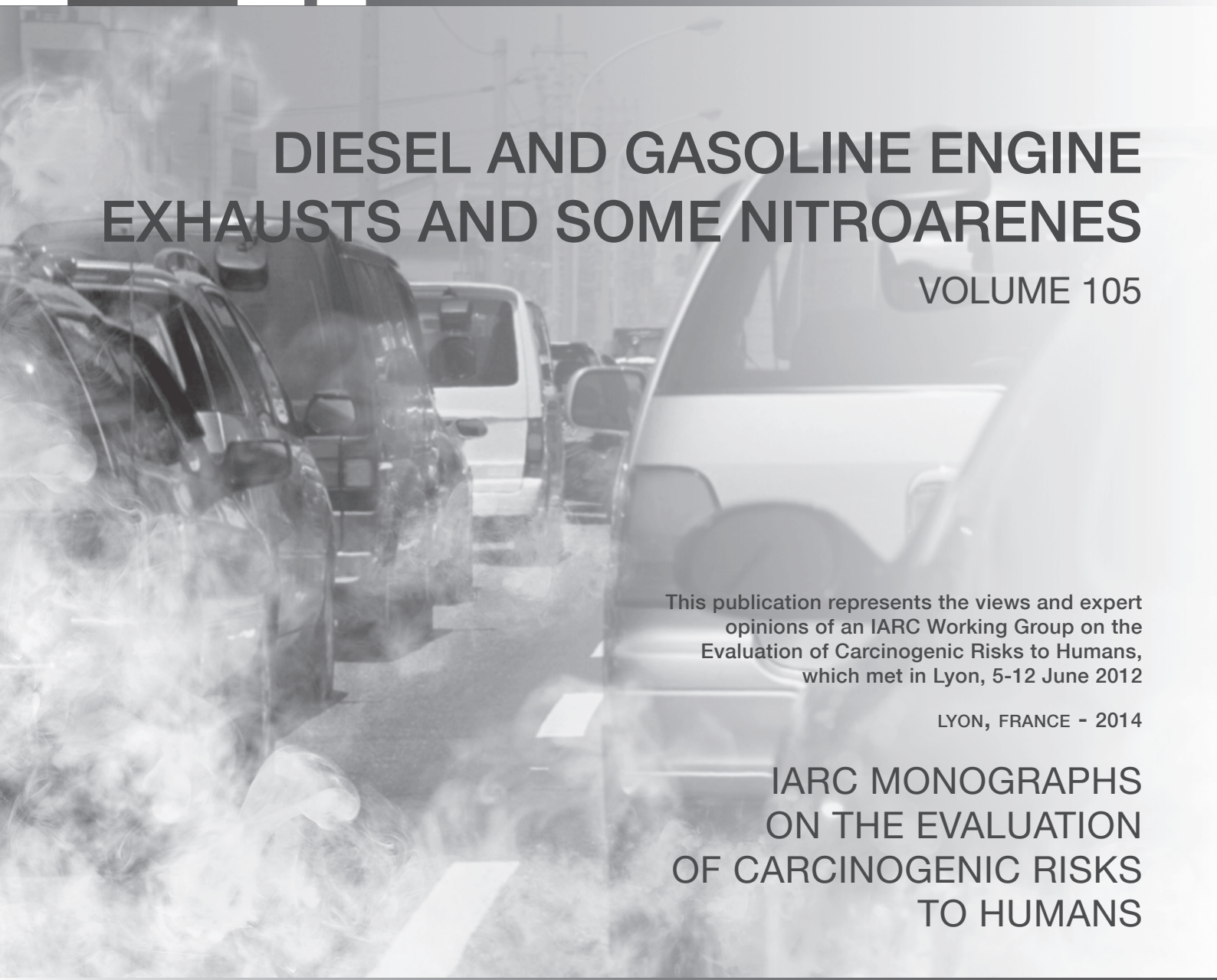

# DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 5-12 June 2012

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

# 1,8-DINITROPYRENE

1,8-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

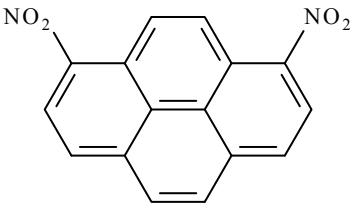
#### *1.1.1 Nomenclature*

*Chem. Abstr. Serv. Reg. No.*: 42397-65-9

*Chem. Abstr. Name*: Pyrene, 1,8-dinitro-

*IUPAC Systematic Name*: 1,8-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); 299–300 °C (Paputa-Peck *et al.*, 1983)

*Spectroscopy data*: Ultraviolet, infrared, 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*

1,8-Dinitropyrene is available for research purposes at 98% purity (Sigma-Aldrich, 2012). The ChemicalBook web site lists five companies that supply 1,8-dinitropyrene (ChemicalBook, 2012). Radiolabelled ($^{14}C$ and $^{3}H$) 1,8-dinitropyrene can be prepared in commercial laboratories.

**Table 1.1 Levels of 1,8-dinitropyrene in diesel engine exhaust particles and their extracts**

| Reference | Vehicle/engine | Concentration of 1,8-DNP (pg/mg particulate matter) |
|---|---|---|
| Nishioka *et al.* (1982) | Passenger cars (LDD) | ND–400[a] |
| Gibson (1983) | Diesel cars 1978–82 (LDD) | 13–25 |
| Nakagawa *et al.* (1983) | Idling 6-tonne bus from 1970 (HDD), 1200 rpm | 3400 |
| Schuetzle & Perez (1983) | Heavy-duty vehicle | |
| | Idle | < 800 |
| | High-speed, no load | 1200 |
| | High-speed, full load | 800 |
| Salmeen *et al.* (1984) | Passenger cars (LDD) | 500–700 |
| Draper (1986) | Commercial mining engine (HDD), 100% load, 1200 rpm | ND (< 290) |
| | Commercial mining engine (HDD), 75% load, 1800 rpm | ND (< 1300) |
| Tokiwa *et al.* (1986) | Idling engine [not further specified] | 13 |
| Hayakawa *et al.* (1992) | Idling engine (LDD) | 128.5[b] |

[a] Range of three different engines
[b] Using a much more sensitive analytical method
DNP, dinitropyrene; 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.

1,8-Dinitropyrene has been reported to be a photosensitizer, and to increase the spectral activity of bis-azide compounds with light (Tsunoda *et al.*, 1973).

## 1.4 Occurrence and environmental exposure

### *1.4.1 Engine exhaust*

The reader is also 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 on diesel engine emissions were performed in the 1980s, and showed a range of concentrations of 1,8-dinitropyrene in the particulate matter (PM) (Table 1.1).

1,8-Dinitropyrene was detected at a level of 3.4 ng/mg in an extract of particles from the exhaust of a heavy-duty diesel engine (Nakagawa *et al.*, 1983). Other investigators have found concentrations of between 0.5 ± 0.3 and 0.7 ± 0.2 ng/mg in extracts (Salmeen *et al.*, 1984), not detected and 0.4 ng/mg in extracts from three different diesel engines (Nishioka *et al.*, 1982), and 0.013 and 0.025 ng/mg in particles (Gibson, 1983) from the exhausts of light-duty diesel engines (reviewed in Fu & Herreno-Saenz, 1999). The production of dinitropyrenes therefore appears to be dependent on engine size and operating conditions.

Hayakawa *et al.* (1994) examined nitroarenes in PM emissions from 15 diesel and gasoline engine vehicles. Compared with diesel engine exhausts, gasoline engine exhausts contained

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

| | No. of samples | Concentration (pg/mg)[a] | | | |
|---|---|---|---|---|---|
| | | 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 |

[a] Values are the means ± standard deviations
DNP, dinitropyrene; NP, nitropyrene
From Hayakawa *et al.* (1992, 1994)

approximately twice the mass concentration of 1,8-dinitropyrene ([102 pg/mg] versus [61 pg/mg]; Table 1.2); however, the ratio of the concentrations of 1,8-dinitropyrene to 1-nitropyrene was 29% for gasoline and 0.5% for diesel engine exhaust, which was assumed to be the result of differences in combustion conditions. The diesel engines produced many more particulates, and their total emissions of dinitropyrene isomers were therefore much greater. In air concentrations of emissions from mixed traffic, the ratio of 1,8-dinitropyrene to 1-nitropyrene decreased as the relative number of diesel vehicles increased.

In the past decade, several types of particulate filters have been developed to filter PM from diesel engine exhaust to control emissions (Heeb *et al.*, 2010). The accumulated soot particles and organic carbon components, including polycyclic aromatic hydrocarbons (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 in the *Monograph* on Diesel and Gasoline Engine Exhausts in this Volume).

In a series of laboratory tests, a range of diesel particulate filters were tested to compare their impact on PAH and nitro-PAH emissions (Heeb *et al.*, 2010). All filters tested, which removed up to 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 at temperatures of 293–573 °K (20–300 °C). The lower temperatures produced more 1-nitropyrene on the filter, 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 affected similarly.

### *1.4.2 Atmospheric particulate matter*

Nitration of pyrene during atmospheric processes leads to the formation of 2- but not 1-nitropyrene, because the oxidants present differ from those involved in combustion, which produces 1-nitropyrene (Pitts, 1987). Thus, dinitropyrenes are not produced by atmospheric processes.

The presence of dinitropyrenes [not further characterized] in respirable particles from ambient atmospheric samples was inferred from mutagenicity testing of polycyclic organic matter extracts (Pitts, 1987). Early sampling data collected at several locations showed a wide range of 1,8-dinitropyrene concentrations (Table 1.3). In remote, rural or unindustrialized areas, the content of 1,8-dinitropyrene in airborne PM was

**Table 1.3 Concentrations of 1,8-dinitropyrene in air samples and collected particulate matter**

| Reference | Sample location<br>Season | Concentration | |
|---|---|---|---|
| | | Particulate matter (pg/mg) | Atmosphere (pg/m³) |
| Tokiwa *et al.* (1983) | Santiago, Chile (urban) | 200 | – |
| Siak *et al.* (1985) | *South-eastern MI, USA* | | |
| | Summer | 0.29–0.61 | 0.023–0.061 |
| Tanabe *et al.* (1986) | Tokyo, Japan | ND–79.3 | ND–6.6 |
| Gibson (1986) | *Bermuda (remote)* | | |
| | Summer | 3.5 | 0.07[a] |
| | Winter | 4.4 | 0.06[a] |
| | *Delaware, USA (rural)* | | |
| | Summer | 2.4 | 0.06[a] |
| | *Warren, MI, USA (suburban)* | | |
| | Winter | < 4 | < 0.10[a] |
| | Summer | 2.1 | 0.13[a] |
| | *Detroit, MI, USA (urban)* | | |
| | Summer | 2.5 | 0.34[a] |
| | *River Rouge, MI, USA (industrial)* | | |
| | Summer | 13.1 | 1.26[a] |
| | *Dearborn, MI, USA (industrial)* | | |
| | Summer | 20 | 3.80[a] |

[a] Calculated by the IARC Working Group (IARC, 1989)
ND, not detected

in the range of 4.6–8.3 pg/mg and the corresponding air levels were 0.12–0.30 pg/m³. In contrast, in heavily industrialized areas, the PM contained 43–46 pg/mg 1,8-dinitropyrene with air concentrations of 4.44–7.50 pg/m³ (Gibson, 1986). The large urban cities of Tokyo, Japan, and Santiago, Chile, had levels of 1,8-dinitropyrene that ranged up to 200 pg/mg (Tokiwa *et al.*, 1983; Tanabe *et al.*, 1986). One study in Michigan, USA, found much lower levels of 1,8-dinitropyrene in total suspended particles than other investigators (Siak *et al.*, 1985).

More recent studies have assessed the concentrations in ambient air of 1-nitropyrene and the 1,3-, 1,6- and 1,8-dinitropyrene simultaneously, and 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 a level of 3.25 ± 0.63 mg/kg of particulate extract.

Gas and liquefied petroleum gas burners are widely used for home heating and cooking. Levels of 1,8-dinitropyrene of 0.88 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 whereby some nitration of pyrene also occurred. A carbon black sample manufactured before 1979 was reported to contain 23.4 mg/kg 1,8-dinitropyrene (Sanders, 1981); another 'long-flow' furnace carbon black sample was also found to contain this compound (Ramdahl & Urdal, 1982). Subsequent changes in the production technique reduced the total extractable nitropyrene content from uncontrolled levels of 5–100 ng/mg to below 0.3 ng/mg (Rosenkranz *et al.*, 1980; Sanders, 1981; Butler *et al.*, 1983). 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 0.16 mg/kg 1,8-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 1,8-dinitropyrene (total dose, 200 nmol [58.7 µg]; purity, > 99%) or benzo[*a*]pyrene (total dose, 560 nmol [140 µg]; purity, > 99%) 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, 31 males and 33 females in the treated group, 37 males and 27 females in the positive-control group and 28 males and 31 females in the vehicle-control group were still alive. All surviving mice were killed after 1 year. In the group injected with 1,8-dinitropyrene, 5 out of 31 (16%) males developed liver tumours compared with 2 out of 28 (7%) controls. 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). [The Working Group noted the short observation period.]

#### *3.1.2 Subcutaneous administration*

A group of 20 male BALB/c mice, aged 6 weeks, received subcutaneous injections of 0.05 mg of 1,8-dinitropyrene (purity, > 99.9%) dissolved in 0.2 mL DMSO (total dose, 1 mg) once a week for 20 weeks. A positive-control group of 20 males received injections of 0.05 mg of benzo[*a*]pyrene, and a further group of 20 mice served as controls. [It was unclear whether the animals were untreated or injected with DMSO.] Animals were observed for 60 weeks or until moribund. After 60 weeks, 6 out of 15 (40%) mice injected with 1,8-dinitropyrene had developed subcutaneous tumours; no such tumours were found in controls ($P < 0.05$). All of the subcutaneous tumours were diagnosed histologically as malignant fibrous histiocytomas [a term used as a specific diagnosis for subcutaneous sarcomas]. Some animals in the 1,8-dinitropyrene-treated group developed tumours of the lung or liver (Otofuji *et al.*, 1987). [The Working Group noted the small number of animals used.]

### 3.2 Rat

See Table 3.2

## Table 3.1 Studies of the carcinogenicity of 1,8-dinitropyrene in mice

| Strain (sex)<br>Duration<br>Reference | Dosing regimen<br>Animals/group at start | Incidence of tumours | Significance | Comments |
|---|---|---|---|---|
| CD1 (M, F)<br>12 mo<br>Wislocki *et al.* (1986) | Intraperitoneal administration<br>0 (control), 200 nmol [58.5 mg] 1,8-DNP or 560 nmol [140 µg] B[*a*]P in 10, 20 or 40 µL DMSO at 1, 8 and 15 d after birth<br>Groups of 90 M, 100 F newborn | Liver (adenoma):<br>M–2/28 (7%), 4/31 (13%), 11/37 (30%)<br>F–0/31, 0/33, 0/27<br>Liver (carcinoma):<br>M–0/28, 1/31 (3%), 7/37 (19%)<br>F–0/31, 0/33, 0/27<br>Lung (adenoma):<br>M–1/28 (4%), 1/31 (3%), 13/37 (35%)<br>F–0/31, 2/33 (6%), 13/27 (48%)<br>Lung (carcinoma):<br>M–0/28, 0/31, 0/37<br>F–0/31, 0/33, 0/27<br>Malignant lymphoma:<br>M–1/28 (4%), 1/31 (3%), 2/37 (5%)<br>F–1/31 (3%), 1/33 (3%), 4/27 (15%) | NS (1,8-DNP) | Purity, > 99%<br>Small number of animals per group and short observation period. |
| BALB (M)<br>60 wks or until moribund<br>Otofuji *et al.* (1987) | Subcutaneous injection<br>0.05 mg 1,8-DNP or 0.05 mg B[*a*]p in 0.2 mL DMSO (total dose, 1 mg), once/wk for 20 wks<br>Groups of 20 aged 6 wks old;<br>20 controls (unclear if injected with DMSO) | Subcutaneous (all tumours):<br>0/13 (control), 6/15 (40%)*, 16/16 (100%)*<br>Lung (all tumours):<br>3/13 (23%), 6/15 (40%), 1/16 (6%)<br>Liver (all tumours):<br>3/13 (23%), 2/15 (13%), 0/16 | *$P < 0.05$ compared with controls | Purity, > 99.9% |

B[*a*]p, benzo[*a*]pyrene; d, day; DMSO, dimethyl sulfoxide; DNP, dinitropyrene; F, female; M, male; mo, month; NS, not significant; wk, week

## Table 3.2 Studies of the carcinogenicity of 1,8-dinitropyrene in rats

| Strain (sex)<br>Duration<br>Reference | Dosing regimen,<br>Animals/group at start | Incidence of tumours | Significance | Comments |
|---|---|---|---|---|
| CD (F)<br>76–78 wks<br>King (1988);<br>Imaida *et al.* (1991) | Oral administration (intragastric intubation)<br>0 or 10 µmol [3 mg]/kg bw in DMSO; (total dose, 16 µmol [4.7 mg]/rat), 3 ×/wk for 4 wks<br>Groups of 36 weanlings | Leukaemia:<br>0/36, 1/36 (3%)<br>Mammary (all tumours):<br>12/35 (34%), 22/36 (61%)*<br>Mammary (adenocarcinoma):<br>5/35 (14%), 12/36 (33%)*<br>Mammary (fibroadenoma):<br>9/35 (26%), 12/36 (33%)<br>Adrenal gland (pheochromocytoma):<br>4/36 (11%), 12/36 (33%)<br>Adrenal gland (cortical adenoma):<br>6/36 (17%), 14/36 (39%)<br>Pituitary gland (carcinoma):<br>2/36 (6%), 13/36 (36%)**<br>Pituitary gland (adenoma):<br>9/36 (25%), 12/36 (33%) | [*$P = 0.05$]<br><br><br><br><br><br>[**$P < 0.005$] | Purity > 99%<br>Study limited by the short duration of both treatment and observation periods and the use of a single dose |
| CD (F)<br>Death 12–15 wks after initial treatment; control group: 76–78 wks<br>King (1988);<br>Imaida *et al.* (1991) | Intraperitoneal administration<br>0 or 10 µmol [3 mg] in DMSO (total dose, 16 µmol [4,7 mg]/rat), 3 × /wk for 4 wks<br>Groups of 33–36 weanlings | Peritoneal cavity (malignant fibrous histiocytoma):<br>0/31, 29/33 (88%)<br>Leukaemia:<br>0/31, 7/33 (21%)<br>Mammary (adenocarcinoma):<br>3/31 (10%), 14/33 (42%)<br>Mammary (fibroadenoma):<br>5/31 (16%), 4/33 (12%) | $P < 0.0001$<br><br>$P < 0.01$<br><br>$P < 0.01$<br><br>NS | Purity, > 90%<br>Study limited by short duration of both treatment and observation periods |
| F344/DuCrj (M)<br>169 d<br>Ohgaki *et al.* (1984) | Subcutaneous injection<br>0 or 0.2 mg in 0.2 mL DMSO (total dose, 4 mg), twice/wk for 10 wks<br>Groups of 10 or 20 aged 6 wks | Injection site (subcutaneous sarcoma):<br>0/20, 10/10 (100%) | [$P < 0.0001$] | Impurities: 0.4% 1,3-dinitropyrene, 0,6% 1,6-dinitropyrene; < 0.05% other nitropyrenes<br>Study limited because of the small number of animals studied, the short treatment and observation periods and the possible influence of the contamination with 1,3-dinitropyrene and 1,6-dinitropyrene |

**Table 3.2 (continued)**

| Strain (sex)<br>Duration<br>Reference | Dosing regimen,<br>Animals/group at start | Incidence of tumours | Significance | Comments |
|---|---|---|---|---|
| F344/DuCrj (M)<br>320 d<br>Ohgaki *et al.*, (1985) | Subcutaneous injection<br>0, 0.02 or 0.002 mg in 0.2 mL DMSO (total dose, 0.04 or 0.4 mg), twice/wk for 10 wks<br>Groups of 10 or 20 aged 6 wks | Injection site (subcutaneous sarcoma):<br>0/20, 9/10 (90%)*, 10/10 (100%)* | *[$P < 0.0001$] | Impurities: 0.4% 1,3-dinitropyrene<br>Study limited because of the small number of animals studied, the short treatment and observation periods and the possible influence of the contamination with 1,3-dinitropyrene. |
| CD (F)<br>Exposure group:<br>164 d;<br>control group:<br>495 d<br>King (1988);<br>Imaida *et al.* (1995) | Subcutaneous injection<br>Suprascapular injection starting within 24 h of birth; 1st dose: 2.5 mol/kg bw;<br>2nd–3rd doses: 5 mol/kg bw;<br>4th–8th doses: 10 mol/kg bw<br>(total dose, 6.3 mmol [1.9 mg] in DMSO), once/wk for 8 wks<br>37, treated group; 40 controls (DMSO) | Injection site (malignant fibrous histiocytoma):<br>0/40, 37/37 (100%)*<br>Leukaemia:<br>0/40, 8/37 (22%)**<br>Mammary (adenocarcinoma):<br>1/40 (3%), 5/37 (14%)<br>Mammary (fibroadenoma):<br>6/40 (15%), 0/46<br>Mammary (adenoma):<br>1/40 (3%), 0/37 | *$P < 0.0001$<br>**[$P < 0.005$] | Purity > 99%<br>Study limited by the short duration of both the treatment and observation periods and the use of a single dose |

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; M, male; NS, not significant; wk, week

### 3.2.1 Oral administration

Groups of 36 female weanling Sprague-Dawley rats received intragastric intubations of 0 (control) or 10 µmol [3 mg]/kg body weight (bw) of 1,8-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. The animals were observed for 76–78 weeks and then killed. One rat (3%) treated with 1,8-dinitropyrene and none of the controls developed leukaemia. The total number of mammary-tumour bearing animals in the 1,8-dinitropyrene-treated group was significantly increased. The incidence of mammary gland adenocarcinoma (12 out of 36; 33%, $P < 0.05$) was significantly higher than that observed in the control rats (5 out of 35; 14%), but that of fibroadenoma in treated rats (12 out of 36; 33%) was not significantly different compared with controls (9 out of 35; 26%). Adrenal gland 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.]

### 3.2.2 Intraperitoneal administration

Groups of 36 female weanling CD rats received intraperitoneal injections of 0 (control) or 10 µmol [3 mg]/kg bw of 1,8-dinitropyrene (purity, > 99%) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) three times a week for 4 weeks (total dose, 16 µmol [4.7 mg]/rat). Treatment with 1,8-dinitropyrene resulted in early deaths 12–15 weeks after the initial treatment. The first intraperitoneal tumour was detected at week 17; 29 out of 33 (88%) of the treated rats developed malignant fibrous histiocytomas of the peritoneal cavity ($P < 0.0001$), and a significantly increased incidence of myelocytic leukaemia (7 out of 33; 21%) was observed in this group ($P < 0.01$). No such malignancies developed among 31 vehicle controls after an observation period of 76–78 weeks. Mammary gland adenocarcinomas were observed in 14 out of 33 (42%) treated animals compared with 3 out of 31 (10%) controls; the difference in the incidence was statistically significant ($P < 0.001$) (King, 1988; Imaida *et al.*, 1991).

### 3.2.3 Subcutaneous administration

Ten male Fischer 344/ DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.2 mg of 1,8-dinitropyrene ([purity unspecified]; impurities: 0.4% 1,3-dinitropyrene, 0.6% 1,6-dinitropyrene and < 0.05% other nitropyrenes) dissolved in 0.2 mL DMSO (total dose, 4 mg) twice a week for 10 weeks. A control group of 20 rats received injections of 0.2 mL DMSO alone. The animals were killed between days 140 and 169. Sarcomas developed at the site of injection in all treated rats between days 113 and 127. No tumours were observed in other organs of treated rats, and no local tumours developed among the control animals (Ohgaki *et al.*, 1984). [The Working Group noted that, while it recognized the contamination of the study material with 1,3- and 1,6-dinitropyrene, the tumour response was so strong that it can be attributed to the exposure to 1,8-dinitropyrene.]

Two groups of 10 male Fischer 344/ DuCrj rats, aged 6 weeks, received subcutaneous injections of 0.002 or 0.02 mg of 1,8-dinitropyrene ([purity unspecified]; impurities: 0.4% 1,3-dinitropyrene; 0.6% 1,6-dinitropyrene and < 0.05% other nitropyrenes) dissolved in 0.2 mL of DMSO (total doses, 0.04 or 0.4 mg) twice a week for 10 weeks. A control group of 20 rats received injections of 0.2 mL of DMSO alone. All treated animals were killed on day 320 and control rats on day 650. Sarcomas developed at the site of injection between days 123 and 156 in all 10 rats treated with 0.4 mg of 1,8-dinitropyrene and between days 213 and 320 in 9 out of 10 (90%) rats treated

with 0.04 mg of 1,8-dinitropyrene. No tumours were observed in other organs of treated rats or at the injection site in control animals (Ohgaki *et al.*, 1985). [The Working Group noted that, while it recognized the contamination of the study material by 1,3- and 1,6-dinitropyrene, the tumour response was so strong that it can be attributed to the exposure to 1,8-dinitropyrene.]

In a lifetime study, a group of 37 female newborn Sprague-Dawley rats received subcutaneous injections into the suprascapular region of 1,8-dinitropyrene (purity, > 99%; total dose, 6.3 µmol [1.8 mg]) dissolved in DMSO (1.7 µmol [0.5 mg]/mL) at weekly intervals starting within 24 hours of birth (first dose, 2.5 mmol/kg bw; second the third doses, 5 mmol/kg bw; doses 4–8, 10 mmol/kg bw). A group of 40 animals injected with DMSO alone served as controls. Average survival was 164 days for treated animals and 495 days for controls. Malignant fibrous histiocytomas developed rapidly at the injection site in treated rats; the first tumour was seen 122 days after the initial injection and, by 20 weeks, all treated rats had developed this tumour (37 out of 37; $P < 0.0001$). In addition, 8 out of 37 treated rats (22%; $P < 0.005$) had leukaemia. Controls developed no such malignancies. Mammary gland adenocarcinomas were also observed in 5 out of 37 (14%) treated rats, although this incidence did not differ significantly from that in the control group (1 out of 40; 3%) (King, 1988; Imaida *et al.*, 1995).

# 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 metabolism of 1,8-dinitropyrene was studied *in vitro* using rat liver and mammary gland cytosols, as well as dog liver cytosols, and in in-vivo systems (IARC, 1989). In conventional CD rats, several metabolites were detected following the oral administration of 1,8-dinitropyrene (1.0 µmol, 0.3 mg): *N*,*N*-diacetyl-1,8-diaminopyrene, 1,8-diaminopyrene, 1-acetylamino-8-nitropyrene and unidentified polar metabolites in the faeces. In germ-free animals, only 1-amino-8-nitropyrene and the polar metabolites were detected.

## 4.2 Genetic and related effects

### 4.2.1 Humans

No data were available to the Working Group.

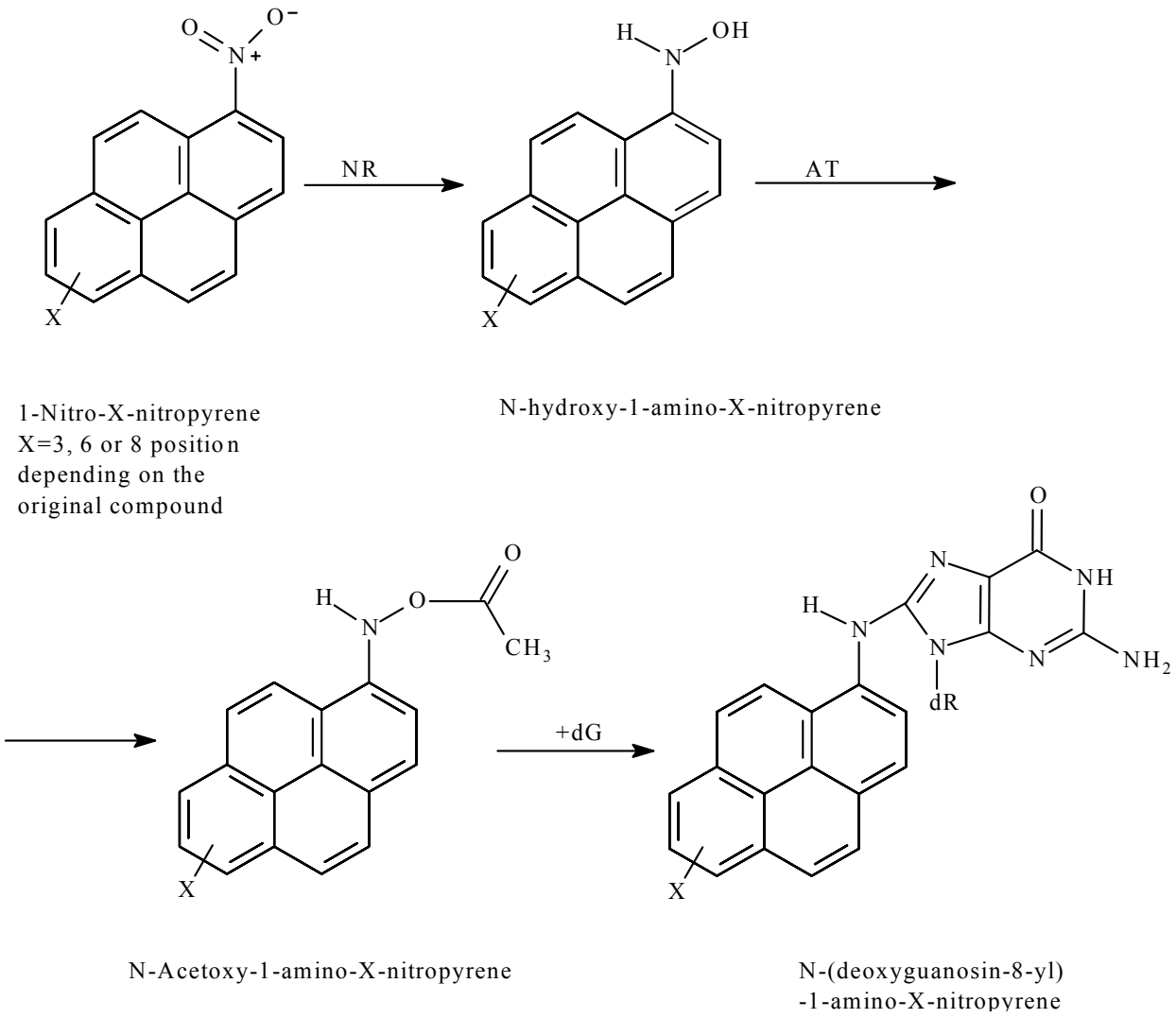
### 4.2.2 Experimental systems

See Fig. 4.1.

In both conventional and germ-free male CD rats, *N*-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene was detected as the major DNA adduct in the liver and mammary gland, but the levels of binding were considerably lower in germ-free animals (IARC, 1989).

The majority of the studies on the genotoxicity of nitro-PAHs have used the *Salmonella typhimurium* microsome assay with a standardized test protocol using several strains (TA98, TA100, TA1535, TA1537 or TA1538). Overall, nitroreductases and *O*-acetyltransferases have been shown to be important enzymes in the mutagenic activation of 1,8-dinitropyrene in bacteria (IPCS, 2003). No significant difference in mutagenicity was seen between nitroreductase-deficient strains, standard strains and nitroreductase-overexpressing strains (IPCS, 2003). 1,8-Dinitropyrene showed stronger mutagenicity in this system in the absence of metabolic activation. The mutagenic activity of 1,8-dinitropyrene

**Fig. 4.1 Metabolic activation pathway and DNA adduct formation**




NR, nitroreductase; AT, acetyltransferase; dG, deoxyguanosin
Compiled by the Working Group

was clearly decreased with *O*-acetyltransferase-deficient strains ($AT^-$) and increased with *O*-acetyltransferase-overproducing strains ($AT^+$) compared with that observed in standard tester strains (IARC, 1989; Tokiwa *et al.*, 1994). These results indicate that *O*-acetylation appears to be a critical mutagenic activation pathway in *S. typhimurium*. Nitrated pyrenes induced primarily frameshift mutations.

The genotoxicity of 1,8-dintropyrene has been reported in *S. typhimurium* and in bacterial systems other than the *Salmonella* microsome assay (IARC, 1989; Mersch-Sundermann *et al.*, 1991, 1992; Oda *et al.*, 1992, 1993; Jurado *et al.*, 1993; Busby *et al.*, 1994; Jurado *et al.*, 1994; Nohmi *et al.*, 1995; Shane & Winston, 1997; Yamazaki *et al.*, 2000); in general, the results, with a few exceptions, were consistent. 1,8-Dinitropyrene induced DNA damage and mutations in several strains of bacteria, and DNA single-strand breaks in mouse hepatocytes, cultured Chinese hamster V79 cells and cultured rat hepatoma

cells, and activated the synthesis of viral DNA in polyoma virus-transformed rat fibroblasts. It induced unscheduled DNA synthesis in mouse and rat hepatocytes, and in rabbit lung Clara and alveolar type II cells. 1,8-Dintropyrene induced mutations in cultured mouse lymphoma and Chinese hamster ovary cells, chromosomal aberrations in Chinese hamster ovary cells and human fibroblasts, chromatid-type chromosomal aberrations in rat epithelial cells, and morphological transformation in Syrian hamster embryo cells. Sarcomas produced in 1,8-dinitropyrene-treated rats contained activated C-$K_i$-*ras* oncogenes (IARC, 1989).

Landvik *et al.* (2007) showed that 1-nitropyrene and dinitropyrenes induced apoptosis in Hepa1c1c7 cells with the following potency: 1,3-dinitropyrene > 1-nitropyrene > 1,8-dinitropyrene. These compounds induced cytochrome P450 (CYP) 1a1 and activated various intracellular signalling pathways related to apoptosis. The most important finding was that the most mutagenic and carcinogenic compound tested in this study, 1,8-dinitropyrene, induced little (if any) cell death, despite the fact that this compound seemed to cause the greatest DNA damage as determined by DNA adduct formation, increased phosphorylation of p53 and accumulation of cells in the S-phase. Immunohistochemical studies revealed that the p53 protein did not accumulate in the nucleus, suggesting that 1,8-dinitropyrene inactivated the pro-apoptotic function of the p53 protein by a non-mutagenic event. Taken together, Landvik *et al.* (2007) suggested that, after exposure to 1,8-dinitropyrene, more cells may survive with DNA damage and thereby increase the mutagenic and carcinogenic potential of the compound.

## 4.3 Other relevant data

No data were available to the Working Group on the acute toxicity of 1,8-dinitropyrene. As previously reported (IARC, 1989), ulcer and scar formation at the site of injection were observed in rats after repeated subcutaneous injection of 0.2 mg per animal. 1,8-Ditropyrene administered intraperitoneally to young male Sprague-Dawley rats (three times at 2.5 mg/kg bw) resulted in increases in the activities of aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-*O*-deethylase, aminopyrine-*N*-demethylase and 1-nitropyrene reductase in the liver microsomes compared with untreated controls.

## 4.4 Mechanistic considerations

To gain insights into the mechanisms by which 1,6-dinitropyrene induced mutations in human cells, Boldt *et al.* (1991) investigated the mutagenic effects of *N*-hydroxy-1-amino-6-nitropyrene, which is derived from the nitroreduction of 1,6-dinitropyrene. The shuttle vector plasmids, pS189, in human 293 cells were exposed to [$^3$H]1-nitro-6-nitrosopyrene for one hour in the presence of ascorbic acid to generate the corresponding hydroxylamine. A linear increase was observed in the number of DNA adducts per plasmid (as a function of applied concentration) and also in the frequency of *supF* mutants (as a function of adducts per plasmid). 1,6-Ditropyrene induced base substitutions, primarily GC→TA transversions, but produced a significant fraction of –1 frameshifts, most of which were located in a unique run of the five Gs in the gene. The 'hot spots' for adduct formation were not perfectly correlated with those for the induction of mutation. Thus, the ultimate biological effect of 1,6-dinitropyrne depends not only on the number of adducts (measured by the $^{32}$P-postlabelling method) originally formed, but also on other processes such as cellular DNA repair, which may remove these adducts from the plasmids before DNA replication occurs, as well as on the structure of the neighbouring bases at the site of adduction. *In vivo*, mutagenesis was reported in the lungs of gpt-delta transgenic mice that received intratracheal instillations of

1,6-dinitropyrene (Hashimoto *et al.*, 2006), and the major mutations induced included G:C→T:A transversions and 1-base deletions.

To understand further the mechanisms that can account for mutagenesis, a commercially available mixture of dinitropyrenes (1,3-, 1,6- and 1,8-dinitropryene and unidentified isomer(s) with contents of 20.2%, 30.4%, 35.2% and 14.2%, respectively) was administered by intragastric intubation in the Muta Mouse model at doses of 200 and 400 mg/kg bw once a week for 4 weeks. Several organs (the liver, lung, colon, stomach and bone marrow) were collected 7 days after the final treatment, and the mutation frequencies of *lacZ* and *cII* genes were analysed. Spontaneous mutation frequencies were in the range of $3.1 \times 10^{-5}$ to $7.6 \times 10^{-5}$ and $1.6 \times 10^{-5}$ to $5.9 \times 10^{-5}$ for the *lacZ* and *cII* genes, respectively. These increases above spontaneous levels were most apparent in the colon, where six- and eightfold increases were observed in the *lacZ* and *cII* genes, respectively. The increase was also evident in the stomach for both genes, although this was not statistically significant at the higher dose for the *cII* gene. A statistically significant increase was observed in the liver and lung for the *lacZ* gene, but was not evident in the liver for the *cII* gene. A fourfold increase was observed in bone marrow for both genes, but was statistically significant only for the *cII* gene. Base-substitution mutations in the colon predominated in both untreated and dinitropyrene-treated mice. The treatment with dinitropyrenes increased the incidence of G:C→T:A transversions and decreased that of G:C→A:T transitions. The G:C→T:A transversions were probably caused by the guanine-C8 adduct (Kohara *et al.*, 2002).

Female Sprague-Dawley rats were given a single intraperitoneal injection of 1,6-dinitropyrene and both covalent DNA adduct formation (*N*-deoxyguanoisin-8-yl)-1-amino-6-nitropyrene) and oxidative DNA damage (5-hydroxymethyl-2′-deoxyuridine and 8-hydroxy-2′-deoxyguanosine) were assessed in the liver, mammary gland, urinary bladder and nucleated blood cells at 3, 12, 24 and 48 hours after treatment (Djurić *et al.*, 1993). The covalent adduct was detected in all tissues and the bladder had the highest levels. The levels of 5-hydroxymethyl-2′-deoxyuridine were highest in the liver and mammary glands. 1,6-Dinitropyrene did not affect the levels of 8-hydroxy-2′-deoxyguanosine. These results suggest that 1,6-dinitropyrene can induce both covalent DNA binding and certain DNA oxidative damage and that both types of DNA damage may contribute to its carcinogenicity in the rat mammary gland.

Tokiwa *et al.* (1999) showed that 8-hydroxy-2′-deoxyguanosine was detected in all 22 cases of carcinoma in human lung tissues. Intratracheal administration of diesel exhaust particles to rodents (without analysis of the organic components) increased the levels of this oxidative lesion. These results suggest that carbonaceous particles, but not mutagens and carcinogens, promote the formation of this lesion and that, as a mechanism, alveolar macrophages may be involved in diesel particle-induced oxidative damage. Murata *et al.* (2004) investigated the extent of oxidative DNA damage induced by 1,3-, 1,6- and 1,8-dinitropyrene in the presence of nicotinamide adenine dinucleotide phosphate (NADPH)-CYP reductase using the $^{32}$P-5′-end-labelled DNA method. The intensity of DNA damage caused by 1,6- or 1,8-dinitropyrene was stronger than that caused by 1,3-dinitropyrene. Further experiments suggest that dinitropyrenes are enzymatically reduced to the corresponding 1-nitro-X(3,6,8)-nitrosopyrene via the nitro-radical anion, and that these nitroso intermediates are further reduced non-enzymatically by NADPH. Subsequently, auto-oxidation of the nitro radical anion resulted in $O^{-}\cdot$ generation leading to DNA damage. These results indicate that both covalent DNA adducts and DNA oxidative damage may contribute to the mutagenic and carcinogenic effects of dinitropyrenes.

SnrA and cnr bacterial nitroreductases have been previously identified in *Salmonella enterica* serovar Typhimurium (Salamanca-Pinzón *et al.*, 2010). Both SnrA and cnr have been purified, and their capacity to activate dinitropyrenes in the Ames test and their kinetic parameters (*Km* and *Vmax*) were examined. 1,3-Dinitropyrene was efficiently activated by cnr, whereas 1,6- and 1,8-dinitropyrene were scarcely activated by either nitroreductase. A good correlation was obtained between the catalytic efficiency (*Vmax*/*Km*) of the purified cnr (but not of SnrA) and the redox potential of the dinitropyrene. These results suggest that factors other than redox potential are involved in the catalytic activity of SnrA.

The activity of hepatic microsomal enzymes in rats pretreated with a series of nitro-PAHs was examined by Chou *et al.* (1987), who found that some of these compounds, including dinitropyrenes, increased the activities of aryl-hydrocarbon hydroxylase, 7-ethoxycoumarin *O*-deethylase and 1-nitropyrene reductase. None of the compounds caused significant increases in epoxide hydrolase or NADP-cytochrome C reductase. Because nitro reduction appears to be important in the metabolic activation of dinitro-pyrenes, the results of this study suggest that chronic exposure to these agents may result in an increase in DNA adduct formation, which in turn could result in increased tumorigenicity.

The treatment of fish hepatoma PLHC-1 cells with 1,6-dinitropyrene resulted in the induction of CYP1A (Jung *et al.*, 2001). The mRNA levels of CYP1A1, -1A2 and -1B1 were determined using the reverse transcription-polymerase chain reaction in various human cell lines treated with several nitro-PAHs, amino PAH derivatives and PAHs (Iwanari *et al.*, 2002). In inducible cell lines, such as human breast cancer MCF-7 cells, the induction profile of chemical specificity was similar for CYP1A1, -1A2 and -1B1, although the extent of induction differed among cell lines and for the CYP isoforms. 1,3-, 1,6- and 1,8-Dinitropyrene slightly induced CYP1 mRNA, but the 1,3- isomer induced a sixfold induction of CYP1A1 mRNA in MCF-7 cells. The cell-specific induction of the CYP1 family was not related to the expression levels of the arylhydrocarbon receptor, aryl hydrocarbon nuclear translocator or estrogen receptors α and β.

Landvik *et al.* (2007) demonstrated that nitro-PAHs induced apoptosis in Hepa1C1C7 cells with the following order: 1,3-dinitro-pyrene > 1-nitropyrene > 1,8-dinitropyrene. These compounds induced CYP1A1, and activated various intracellular signalling pathways related to apoptosis. 1,3-Dinitropyrene and 1-nitropyrene induced concentration-dependent lipid peroxidation. 1,3-Dinitropyrene caused pro-apoptotic events (increased phosphorylation and accumulation of p53 in the nucleus, cleavage of bid and of caspases 8 and 3, down-regulation of bcl-$X_L$ and phosphorylation of p38 and c-Jun N-terminal kinase/mitogen-activated protein kinase). It also increased the activation of survival signals (phosphorylation of AKt and inactivation [phosphorylation] of pro-apoptotic bad). 1,8-Dinitropyrene induced little (if any) cell death, despite the fact that this compound seemed to induce the greatest DNA damage (as determined by DNA adduct formation, increased phosphorylation of p53 and accumulation of cells in the S-phase). Immunohistochemical analysis revealed that the p53 protein did not accumulate in the nucleus, suggesting that 1,8-dinitro-pyrene inactivated the pro-apoptotic function of p53 by a non-mutagenic event. On the basis of these results, these investigators suggested that, following exposure to 1,8-dinitropyrene, more cells may survive with DNA damage and thereby increase its mutagenic and carcinogenic potential.

Mutagenic activity was evaluated using the umuC test in the presence and absence of metabolic activation (Bonnefoy *et al.*, 2012). The umuC test is based on the induction of the umuC gene as part of the *Salmonella typhimurium*

TA1535 [pSK1002] SOS response to genotoxic lesions induced by xenobiotics. To evaluate the genotoxic effects further, these authors used both the cytokinesis-blocked micronucleus assay and fluorescent *in situ* hybridization of human pan-centromeric DNA probes on human lymphocytes. 1,3-, 1,6-, and 1,8-Dinitropyrene were mutagenic in the umuC test and the effect was dose-dependent in the presence and absence of metabolic activation; however, some exceptions were evident. 1,3- and 1,6-Dinitropyrene induced a clear but statistically non-significant increase in micronucleated cells. However, a significant induction of micronucleated cells was observed for 1,8-dinitropyrene at 0.5 μg/mL. The percentages of observed centromere-negative micronuclei were 63.2% and 81.8% for 1-nitropyrene and 1,8-dinitropyrene, respectively; the corresponding values for 1,3- and 1,6-dinitropyrene were 56.3% and 58.3%, respectively. 1,3- and 1,6-Dinitropyrene exhibited both clastogenic and aneugenic activities but 1,8-dinitropyrene exhibited a dominant clastogenic mechanism.

In summary, 1,6- and 1,8-dinitropyrene are more powerful mutagens in bacterial systems and mammalian systems than 1,3-dinitropyrene. Moreover, their carcinogenic activities exceed those of 1,3-dinitropyrene and 1-nitropyrene. The activation of dinitropyrenes occurs by nitroreduction of one nitro group initially to form nitroso intermediates that are then converted to the corresponding hydroxylamino derivatives. 1,3-Dinitropyrene and 1-nitropyrene were reduced to a much lesser extent than 1,6- and 1,8-dinitropyrene. The mutagenicity of dinitropyrene is related to the ability of the corresponding hydroxylamino derivative to bind to DNA. *O*-Acetylation of the *N*-hydroxylamino group is followed by removal of the acetyl group to yield the active electrophilic nitronium ion that reacts with deoxyguanosine at the C8 position to form *N*-(deoxyguanosine-8-yl)-1-amino-X(3,6,8) nitropyrene. Mutations in the *K-ras* oncogene and *p53* tumour-suppressor gene were observed in 1,6-dinitropyrene-induced lung tumours in rats. 1,8-Dinitropyrene induced greater DNA damage than 1,3-dinitropyrene and 1-nitropyrene but less, if any, cell death in Hepa1c1c cells; this result suggested that, following exposure to 1,8-dinitropyrene, more cells may survive with DNA damage, and thereby increase its mutagenic and carcinogenic activities.

## 5. Summary of Data Reported

### 5.1 Exposure data

1,8-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 1,8-dinitropyrene in the range of 0.1–10% relative to the 1-nitropyrene content in diesel and gasoline exhaust particles and ~1% in airborne particulate matter. 1,8-Dinitropyrene is present in the 1–10 ng/g range in airborne particulate matter collected from ambient atmospheric samples. Air concentrations clearly decline from values in the 0.1–10 pg/m$^3$ range at urban locations to values in the 0.01–0.1 pg/m$^3$ range at suburban locations.

1,8-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

1,8-Dinitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and one study by subcutaneous injection, and in rats in one study by oral administration, one study by intraperitoneal injection and three studies by subcutaneous injection. In mice, intraperitoneal injection of 1,8-dinitropyrene into newborns did not produce an increase in the incidence of tumours at any site that was significantly different from that in controls; the study of the subcutaneous injection of 1,8-dinitropyrene was inadequate to evaluate carcinogenicity. In rats, intragastric administration of 1,8-dinitropyrene caused a significant increase in the incidence of mammary tumours and pituitary carcinomas in females; intraperitoneal injection caused a significant increase in the incidence of malignant histiocytomas of the peritoneal cavity, mammary adenocarcinoma and myelocytic leukaemia in females; and subcutaneous injection of 1,8-dinitropyrene induced injection-site sarcomas in males in two studies and a significant increase in the incidence of malignant histiocytomas and leukaemia in females in one study.

## 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 1,8-dinitropyrene in humans. The metabolism of 1,8-dinitropyrene was investigated in rat liver and mammary gland cytosols *in vitro*. After oral administration to rats, 1,8-dinitropyrene produced the metabolites *N*,*N*-diacetyl-1,8-diaminopyrene, 1,8-diaminopyrene and 1-acetylamino-8-nitropyrene. Studies with nitroreductase/acetyltransferase-deficient or -overproducing strains of bacteria revealed that *O*-acetylation is probably the critical mutagenic activation pathway in *Salmonella*. 1,8-Dinitropyrene induced little, if any, cell death, despite the induction of extensive DNA damage, DNA adduct formation, phosphorylation of *Tp53* and enhanced cell proliferation. A greater number of cells may thus survive with DNA damage, and thereby increase the mutagenic and carcinogenic potential of 1,8-dinitropyrene. Sarcomas in rats treated with 1,8-dinitropyrene contained activated K-*ras* oncogenes.

Overall, these data provide *moderate mechanistic evidence* to support the carcinogenicity of 1,8-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 1,8-dinitropyrene.

## 6.3 Overall evaluation

1,8-Dinitropyrene is *possibly carcinogenic to humans (Group 2B)*.

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