IARC MONOGRAPHS

World Health Organization

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

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

4-NITROPYRENE

4-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.
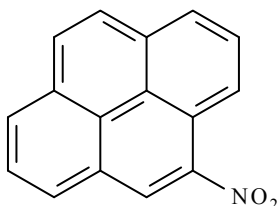
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 57835-92-4

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: Slender orange needles (Bavin, 1959)

Melting-point: 190–192 °C (Paputa-Peck et al., 1983); 196–197.5 °C (Bavin, 1959)

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

Vapour pressure: 4.4×10^{-6} Pa at 25 °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.m³/g/mol at 25 °C (Yaffe et al., 2001)

Spectroscopy data: Ultraviolet and nuclear magnetic resonance data have been reported (Paputa-Peck et al., 1983).

Solubility: Soluble in water (0.017 mg/L) (Yaffe et al., 2001)

1.1.4 Technical products and impurities

One company in Japan produces 4-nitropyrene for analytical or reference purposes, with a purity of > 97%.

Table 1.1 Concentrations of 4-nitropyrene in airborne particulate matter[a]

SRM	Characterization	Year of collection	Location	Content (in ng/g ± 95% CI)		
				4-NP	1-NP	2-NP
1648	Urban area	1978	St. Louis, MO, USA	9.1 ± 0.7	155 ± 29	48.9 ± 2.4
1649a	Urban area	1970s	Washington, DC, USA	5.5 ± 0.6	71.5 ± 5.1	24.4 ± 4.0
BALT. PM-2.5	Airborne particulate matter (PM-2.5 fraction)	1998–90	Baltimore, MD, USA	8.8 ± 1.2	196 ± 3	35.5 ± 5.8
2975	Diesel exhaust particulate matter collected from industrial forklift	NR	NR	173 ± 11	39640 ± 590	< 4[b]
1975	Diesel exhaust particulate extract	NR	NR	68.2 ± 2.4	16070 ± 3	< 4[b]
1650a	Diesel exhaust particulate matter collected from a heat exchanger	NR	NR	135 ± 8	18330 ± 340	< 4[b]

[a] Based on gas chromatography-negative ion chemical ionization-mass spectrometry analysis
[b] Below the limit of detection
CI, confidence interval; NP, nitropyrene; NR, not reported; PM, particulate matter; SRM, standard reference material
From Bamford *et al.* (2003)

1.2 Analysis

Further details on the air sampling and analysis of nitroarenes can be found in Section 1.2 of the *Monographs* on Diesel and Gasoline Engine Exhaust or 1-Nitropyrene in this Volume.

4-Nitropyrene can be extracted from particulate matter with dichloromethane. A method for the determination of 4-nitropyrene by gas chromatography (GC) and chemiluminescence detection has been described (Yu *et al.*, 1984). Bamford *et al.* (2003) and Albinet *et al.* (2006) separated 1-, 2- and 4-nitropyrene by purification with liquid chromatography and solid-phase extraction on alumina and silica columns, followed by GC with negative ion chemical ionization-mass spectrometry (GC-NICI-MS) on a 5% or 50% phenyl-substituted methylpolysiloxane capillary column. [^{2}H$_9$]-Nitrofluoranthene was used as an internal standard.

1.3 Production and use

4-Nitropyrene of unspecified purity is manufactured by one company each in Germany, Norway and the People's Republic of China.

4-Nitropyrene is not produced for purposes other than use in chemical analysis and scientific research.

1.4 Occurrence and exposure

4-Nitropyrene is assumed to be formed by photochemical conversion in the atmosphere (Atkinson *et al.*, 1991), although this would occur through a very slow two-step reaction mechanism (Murahashi *et al.*, 1999).

Murahashi *et al.* (2001) identified 4-nitropyrene (together with seven other nitroarenes) in precipitation water samples collected on the roof of a four-storey building in a residential area and in airborne particulates in Kanazawa, Japan, in September–October 1996, October–November 1996 and in February 1997. [The authors included a chromatogram that showed a peak corresponding to 0.030 pmol/L [7.42 pg/L] in precipitation water samples, but no chemiluminescence spectrum.] In air samples, the nitroarenes were present at a concentration range of 2–1400 ng/g of particulate matter. The abundance of the compounds was 1-nitropyrene

> 2-nitrofluoranthene > 6-nitrochrysene > 2-nitropyrene > 1,3-, 1,6- and 1,8-dinitropyrenes in all three samples.

Albinet *et al.* (2007) reported air concentrations of 4-nitropyrene in Marseille, France. In urban locations, a median of 1.4 pg/m³ and a range of 0.7–2.6 pg/m³ of 4-nitropyrene were observed. In a suburban location, the median and range were 0.6 pg/m³ and 0.1–1.2 pg/m³, respectively. In rural locations, the concentrations were below 0.1 pg/m³.

The presence of 4-nitropyrene was confirmed in the certified standard reference material (SRM) 1649a, an update of SRM1649 produced from airborne particulate matter collected using a baghouse collector in an urban area in Washington, DC, USA, in the late 1970s (NIST, 2001).

The contents of 1-, 2- and 4-nitropyrene in several SRMs are presented in Table 1.1.

In 2003, Bamford *et al.* (2003) reported a value of 5.5 ± 0.6 ng/g of particulate matter when SRM1649a was analysed in triplicate by GC-NICI-MS. In this analysis, 4-nitropyrene was not available as a reference standard and the calibration was based on the response factor of 3-nitrofluoranthene. Using the same technique, Albinet *et al.* (2006) reported a similar value of 6.0 ± 0.9 ng/g in SRM1648a. In these samples, the concentrations of 4-nitropyrene were much higher in diesel exhaust particles than in airborne particulate matter.

Newly developed catalytic diesel particulate filters have been shown to reduce the concentration of 4-nitropyrene in diesel emissions by 40–60% (Heeb *et al.*, 2008, 2010).

1.5 Regulations and guidelines

No regulations or guidelines for 4-nitropyrene were found by the Working Group.

2. Cancer in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

Intraperitoneal administration

Male and female newborn CD-1 mice received three intraperitoneal injections of 4-nitropyrene (purity, > 99%) in dimethyl sulfoxide (DMSO) on day 1 (400 nmol), day 8 (800 nmol) and day 15 (1600 nmol) after birth (total dose, 2800 nmol [692 µg]/mouse). Control mice received either three injections of DMSO with the same dose schedule or a single injection of 560 nmol [140 µg] of benzo[*a*]pyrene (purity, > 99%; vehicle controls). At 25–27 days, mice were weaned and separated according to sex. All surviving mice were killed after 1 year. In males, hepatocellular adenoma or carcinoma (combined) developed in 24 out of 29 (83%; four adenomas, 20 carcinomas) treated mice and 7 out of 28 (25%) controls [$P < 0.005$]. In females, no difference in the incidence of liver tumours was found. The incidence of hepatocellular carcinoma was 20 out of 29 (69%) treated males and 0 out of 30 controls [$P < 0.005$]. Lung tumours occurred in 11 out of 29 (38%; 10 adenomas, one carcinoma) treated males and in 1 out of 28 (4%) male controls [$P < 0.002$], and in 9 out of 29 (31%) treated females and 0 out of 31 female controls [$P < 0.001$] (Wislocki *et al.*, 1986).

3.2 Rat

See Table 3.2

Table 3.1 Study of the carcinogenicity of 4-nitropyrene in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
CD-1 (M, F) up to 52 wk Wislocki *et al.* (1986)	Intraperitoneal injection 0 (control) or 2800 nmol/ mouse in DMSO (400 nmol at 24 h after birth, 800 nmol on d 8 and 1600 nmol at d 15) 28–31 animals/group	Liver (hepatocellular adenoma or carcinoma combined): M–7/28 (25%), 24/29 (83%)* F–0/31, 2/29 (7%) Liver (hepatocellular adenoma): M–2/28 (7%), 4/29 (14%) F–0/31, 1/29 (3%) Liver (hepatocellular carcinoma): M–0/28, 20/29 (69%)* F–0/31, 1/29 (3%) Lung (adenoma or carcinoma combined): M–1/28 (4%), 11/29 (38%)** F–0/31, 9/29 (31%)***	*[$P < 0.005$] **[$P < 0.002$] ***[$P < 0.001$]	Purity, > 99%

d, day; DMSO, dimethyl sulfoxide; h, hour; F, female; M, male; wk, week

3.2.1 Subcutaneous administration

A group of female newborn Sprague-Dawley rats [initial number unspecified] received an initial suprascapular injection of 2.5 μmol/ kg body weight (bw) of 4-nitropyrene (purity, > 99.9%) in DMSO within 24 hours of birth, followed by two weekly injections of 5 μmol/ kg bw and eight weekly injections of 10 μmol/ kg bw (total dose, 75 μmol). Another group of rats received DMSO alone. Animals were killed when moribund or at 86 weeks. A statistically significant increase ($P < 0.005$) in the number of total mammary tumours was observed in the treated group (20 out of 27, 74%; 18 adenocarcinomas, 14 fibroadenomas; induction period, 262 days) compared with controls (17 out of 47, 36%). The incidence of mammary gland adenocarcinoma in treated animals was significantly increased (18 out of 27, 67%, versus 3 out of 47, 6%). The incidence of malignant fibrous histiocytoma (10 out of 27, 37%; $P < 0.001$), leukaemia (5 out of 27, 18%; $P < 0.005$) and Zymbal gland carcinoma (4 out of 27, 14%; $P < 0.05$) was also significantly increased compared with controls (0 out of 47). In the same study, similarly treated

Fischer 344 rats showed no differences in the incidence of mammary or other tumours (King, 1988; Imaida *et al.*, 1995).

3.2.2 Intraperitoneal administration

Groups of 30 weanling Sprague-Dawley rats, aged 30 days, received intraperitoneal injections of 0 or 67 μmol/kg bw of 4-nitropyrene (solution of 25 μmol) dissolved in 1 mL DMSO three times a week for 4 weeks (total dose, 119 μmol). The surviving rats were killed 61 weeks after the first injection. The incidence of total mammary tumours (fibroadenoma, adenoma or adenocarcinoma) in treated rats was significantly increased (17 out of 29, 59%; $P < 0.005$) compared with vehicle controls (4 out of 29, 14%). The incidence of adenocarcinoma was also significantly increased (13 out of 29, 45%; $P < 0.005$) compared with controls (1 out of 29, 3%) (King, 1988; Imaida *et al.*, 1991).

Table 3.2 Studies of the carcinogenicity of 4-nitropyrene in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CD (F) 86 wk King (1988), Imaida et. al. (1995)	Subcutaneous (suprascapular) injection 0 (control) or 75 µmol/kg bw (total dose) in DMSO (2.5 µmol/kg bw at 24 h after birth, 5 µmol/kg bw once/wk for 2 wk, and 10 µmol/kg bw once/wk for 5 wk) Numbers at start unspecified	Mammary gland (total tumours[a]): 17/47 (36%), 20/27 (74%)	$P < 0.005$	Purity, > 99.9% by HPLC
		Mammary gland (adenocarcinoma): 3/47 (6%), 18/27 (67%)	$P < 0.05$	
		Mammary gland (fibroadenoma): 16/47 (34%), 14/27 (52%)	NS	
		Soft tissue (malignant fibrous histiocytoma): 0/47, 10/27 (37%)	$P < 0.001$	
		Leukaemia: 0/47, 5/27 (18%)	$P < 0.005$	
		Zymbal gland (carcinoma): 0/47, 4/27 (14%)	$P < 0.05$	
CD (F) 61 wk King (1988), Imaida et al. (1991)	Intraperitoneal injection 0 or 67 µmol/kg bw in 25 µmol/mL DMSO, 3 ×/ wk for 4 wk (total dose, 119 µmol) 30 animals/group	Mammary gland (total tumours[a]): 4/29 (14%), 17/29 (59%)	$P < 0.005$	Purity NR, synthesized
		Mammary gland (adenocarcinoma): 1/29 (3%), 13/29 (45%)	$P < 0.005$	
CD (F) up to 77 wk King (1988), Imaida et al. (1991)	Direct injection into the mammary gland 0 (control) or 2.03 µmol in 0.1 mL DMSO/rat, directly into 3 left thoracic gland nipple area (d 1) then 3 left inguinal nipple area (d 2) (total dose, 12.3 µmol/rat); inner control, 0.1 mL DMSO into the right side of the same rats 30 animals/group	All tumours[b]: 15/28 (53%), 24/28 (86%)	$P < 0.025$	Purity NR
		Mammary gland (total tumours[a]): 7/28 (25%), 23/28 (82%)	$P < 0.001$	
		Mammary gland (fibroadenoma): 5/28 (18%), 15/28 (54%)	$P < 0.01$	
		Mammary gland (adenocarcinoma): 1/28 (4%), 19/28 (68%)	$P < 0.001$	

[a] Fibroadenoma, adenoma or adenocarcinoma combined

[b] One pituitary adenoma, five pituitary adenocarcinomas, one thyroid adenocarcinomas, one Zymbal gland carcinomas, one adenocortical adenoma, four malignant fibrous histiocytomas, two cutaneous fibromas, one pituitary adenoma, 11 pituitary adenocarcinomas

bw, body weight; d, day; DMSO, dimethyl sulfoxide; F, female; h, hour; HPLC, high-performance liquid chromatography; M, male; NR, not reported; NS, not significant; wk, week

3.2.3 Direct injection into the mammary gland

Groups of 30 female Sprague-Dawley rats, aged 30 days, received injections of 2.03 µmol of 4-nitropyrene in 0.1 mL DMSO each directly into three left thoracic gland nipple areas (on day 1) and then into three inguinal gland nipple areas (day 2) (total dose, 12.3 µmol/rat). As an internal control, 0.1 mL DMSO alone was injected into the right side of the nipple area of the same rats. A separate control group received injections of 0.1 mL DMSO alone into both the left and right side nipple areas (12 injections per rat). Thereafter, rats were observed for up to 77 weeks. The incidence of all tumours, total mammary tumours, mammary fibroadenoma and mammary adenocarcinoma (24 out of 28, 86%; 23 out of 28, 82%; 15 out of 28, 54%; and 19 out of 28, 68%; respectively) in the treated rats was significantly greater than that in their respective controls (15 out of 28, 53%; $P < 0.025$; 6 out of 28 [7 out of 28, 25%; personal communication of the author]; $P < 0.001$; 5 out of 28, 18%; $P < 0.01$; and 1 out of 28, 4%; $P < 0.001$; respectively) (King, 1988; Imaida et al., 1991).

4. Mechanistic and Other Relevant Data

4-Nitropyrene is a constituent of diesel exhaust and is not an atmospheric transformation product; however, because of its concentration and mutagenic potency, it can contribute significantly to the mutagenicity of urban air (Murahashi et al., 1999). As reported in the previous Monograph (IARC, 1989), subcutaneous exposure of newborn female CD rats to 4-nitropyrene produced mammary tumours, and intraperitoneal exposure of newborn mice produced liver tumours in males and lung tumours in males and females. Studies published that have been studied since that time are reviewed here.

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Upadhyaya et al. (1994) showed that 4-nitropyrene was metabolized by 3-methylcholanthrene-induced Sprague-Dawley rat liver microsomes or by a rat-liver metabolic activation system to two primary metabolites, one of which was identified as 4-nitropyrene-9,10-dione. This differs considerably from the in-vitro metabolism of 1-nitropyrene, which yields several phenolic derivatives and trans-dihydrodiols, together with other major metabolites. The major metabolite of 4-nitropyrene in the presence of the epoxide hydrolase inhibitor, 3,3,3-trichloropropylene-1,2-oxide, was 9,10-epoxy-9,10-dihydro-4-nitropyrene. This K-region epoxide may play a role in the genotoxicity of 4-nitropyrene, because such epoxides contribute to the mutagenicity of other nitropyrenes, such as 1-nitropyrene.

Upadhyaya et al. (1994) also showed that, 48 hours after oral administration of [³H]4-nitropyrene to female Sprague-Dawley rats, 32% of the dose was excreted in the urine and 30.6% in the faeces. Compounds in the faeces were identified as the metabolites 4-aminopyrene and 9(10)-hydroxy-4-(acetylamino)pyrene, and 4-nitropyrene itself. The urinary metabolites comprised sulfates and glucuronides of 9(10)-hydroxy-4-(acetylamino)pyrene. Thus, similarly to 1-nitropyrene, 4-nitropyrene is metabolized in vivo via nitroreduction, ring oxidation and a combination of both pathways; however, the pattern of excretion of these two nitropyrenes differs. Approximately 40% of the dose of 4-nitropyrene and its metabolites was found in the urine 168 hours after administration, whereas this value was only 20% for

1-nitropyrene. Thus, the position of the nitro group on the pyrene affects the excretion pattern, and this may play some role in the differences in carcinogenicity between the two compounds. A higher level of 4-nitropyrene metabolites in the urine indicates a higher level in the blood and, thus, more are delivered to the target organ (mammary tissue). Consistent with this is the observation that 4-nitropyrene is a more potent mammary carcinogen than 1-nitropyrene.

A comparative study of the metabolism of [3H]1- and 4-nitropyrene after intraperitoneal injection into female CD rats (Chae et al., 1997) showed that neither the excretion patterns nor the metabolite profiles readily explained why 4-nitropyrene is a more potent mammary carcinogen than 1-nitropyrene (see Section 3 of this *Monograph*). However, high-performance liquid chromatographic analysis of hydrolysates of liver DNA found that only 4-nitropyrene yielded putative multiple DNA adducts, whereas 1-nitropyrene produced none. In addition, 4-nitropyrene bound to mammary DNA at a rate 3.5 times higher than 1-nitropyrene, which might account for the greater potency of 4-nitropyrene as a mammary carcinogen in relation to 1-nitropyrene.

Chae et al. (1999a) examined the ability of microsomes from 15 human livers and 8 human lungs to metabolize 1-, 2- and 4-nitropyrene. All of the liver microsomes produced qualitatively similar metabolites, which were also similar to those produced by all of the lung microsomes; however, the levels of metabolites produced by the lung were lower than those produced by the liver. Ring-oxidized metabolites (phenols and *trans*-dihydrodiols) were produced by all three nitropyrenes, but the nitroreductive metabolism that leads to the formation of aminopyrene was observed only with 4-nitropyrene. The authors concluded that most of the liver microsomal metabolism of 1- and 4-nitropyrene was due to cytochrome P450 (CYP) 3A4, but they could not rule out a minor role for CYP1A2. CYP3A4 metabolized 4-nitropyrene to *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene, 9(10)-hydroxy-4-nitropyrene and 4-aminopyrene.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) DNA adduct formation

DNA adducts (determined by ^{32}P-postlabeling/thin-layer chromatography) were induced in three cell lines treated with 4-nitropyrene: NCI-H322 cells that are derived from a human lung tumour, V79NH cells that are derived from Chinese hamster lung and isolated hepatocytes from Wistar rats (Topinka et al., 1998). Pretreatment of either the NCI-H322 cells or hepatocytes with an inducer of CYP1A1, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, reduced the levels of 4-nitropyrene-induced DNA adducts by nearly 100-fold or to non-detectable levels, respectively. This inactivation of 4-nitropyrene by increased CYP1A1 was probably due to an oxidative attack on the aromatic ring leading to the formation of nitrophenols and conjugates, which are unable to form DNA-binding metabolites.

High-performance liquid chromatographic analysis of hydrolysates of liver and mammary tissue DNA obtained from female CD rats 24 hours after intraperitoneal exposure to [3H]4-nitropyrene showed that the DNA adducts from these tissues co-eluted with those derived from in-vitro incubations of 4-nitropyrene that used a nitroreductive pathway (Chae et al., 1999b).

Zhou & Cho (1998) synthesized *N*-(deoxyguanosin-8-yl)-4-aminopyrene, which is a predicted *N*-(deoxyguanosin-8-yl) adduct of 4-nitropyrene, and found that it had an *anti*-glycosyl conformation with C2'-*endo*(S) sugar

puckering and a nearly planar conformation at the central amine nitrogen; it also had a substitution adjacent to a fused aromatic ring. The authors considered that this adduct was probably responsible for much of the mutagenicity and carcinogenicity of 4-nitropyrene.

(b)   DNA damage

4-Nitropyrene induced DNA adducts in rats (Chae et al.,1997) and in rat hepatocytes *in vitro* (Topinka *et al.*, 1998), and nitroreduction was shown to be responsible for the formation of adducts in the rat mammary gland (Chae *et al.*, 1999b).

(c)   Mutagenicity

4-Nitropyrene induced DNA damage in the *Bacillus subtilus* rec assay (Horikawa *et al.*, 1986), and was mutagenic in a forward mutation assay for resistance to 8-azaguanine in *Salmonella typhimurium* TM677; it was ~10 times more potent in the absence than in the presence of an exogenous metabolic activation system (Busby *et al.*, 1994a). 4-Nitropyrene was also mutagenic in a forward mutation assay at the thymidine kinase +/– locus in human B-lymphoblastoid (MCL-5) cells that express CYP1A1, 1A2, 2A6, 2E1 and 3A4, as well as microsomal epoxide hydrolase (Busby *et al.*, 1994b). A structure–activity study based on the mutagenicity of 4-nitropyrene in *S. typhimurium* TA98 in the presence of an exogenous metabolic activation system found that its mutagenic potency could not be explained fully by the orientation of the nitro group relative to the aromatic plane (Onchoke *et al.*, 2004).

4.3   Other relevant data

The ability of 4-nitropyrene to induce cytogenetic effects, alterations in oncogenes or tumour-suppressor genes, or alterations in gene expression has not been reported.

4-Nitropyrene did not induce cell transformation in isolated rat tracheal cells exposed *in vitro* (West & Rowland, 1994).

4.4   Mechanistic considerations

In addition to the two studies reviewed in the previous *Monograph* (IARC, 1989), which showed that 4-nitropyrene induced tumours in newborn rats and mice, an additional study has confirmed that subcutaneous exposure of female CD rats to 4-nitropyrene induces mammary adenocarcinomas, as well as malignant fibrous histiocytomas (Imaida *et al.*, 1995).

The age-specific carcinogenicity of 1-nitropyrene has been ascribed to the enhanced ratio of nitroreductase to CYP activity in the newborn compared with adults. As noted above, the reductive metabolic pathway for 4-nitropyrene has been shown to produce the 4-nitropyrene-associated DNA adducts found *in vivo* in rodents. Moreover, the *N*-(deoxyguanosin-8-yl)-aminopyrene adducts of 4- and 1- nitropyrene are structurally similar (Onchoke *et al.*, 2004). Thus, 1- and 4-nitropyrene may undergo similar metabolism, which results in mutagenic DNA adducts. In comparison with 1-nitropyrene, the greater mutagenic potency of 4-nitropyrene in bacterial and mammalian cells, as well as its greater carcinogenic potency in rodents, may be due to the higher levels of its metabolites in the blood (Upadhyaya *et al.*, 1994), which may result in the delivery of more mutagenic 4-nitropyrene metabolites than 1-nitropyrene metabolites to the target tissue (the mammary gland).

Similarly to 1-nitropyrene (see Section 3 of the *Monograph* on 1-Nitropyrene in this Volume), 4-nitropyrene may not be carcinogenic in adult rodents; however, no studies in adult rodents have been reported.

5. Summary of Data Reported

5.1 Exposure data

4-Nitropyrene has been detected in the particulate phase of diesel exhaust emissions. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. 4-Nitropyrene has not been detected in gasoline engine exhaust or emissions from other products or processes. Recently, it was identified as a constituent of airborne particulate matter. Concentrations of 4-nitropyrene in outdoor air were in the picogram per cubic metre range and were higher in urban and suburban locations than in rural locations.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Nitropyrene was tested for carcinogenicity in mice in one study by intraperitoneal injection and in rats, in one study by subcutaneous injection, in one study by intraperitoneal injection and in one study by direct injection to the mammary gland area. In mice, intraperitoneal injection of 4-nitropyrene into newborns caused a significant increase in the incidence of liver carcinomas in males and of lung tumours in males and females. In rats, subcutaneous injection of 4-nitropyrene into newborns caused a significant increase in the incidence of mammary tumours, malignant fibrous histiocytomas, leukaemia and Zymbal gland carcinomas; intraperitoneal injection resulted in a significant increase in the incidence of mammary tumours; and direct injection into the mammary gland area produced a significant increase in the incidence of mammary tumours.

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 4-nitropyrene in humans. When administered orally to rats, 4-nitropyrene was converted to 4-aminopyrene and 9(10)-hydroxy-4-(acetylamino)pyrene. Metabolic conversion to 4-nitropyrene-9,10-dione has also been shown *in vitro* in the presence of induced rat-liver microsomes. In the presence of an epoxide hydrolase inhibitor, a major metabolite of 4-nitropyrene was 9,10-epoxy-9,10-dihydro-4-nitropyrene, a K-region epoxide. Most of the metabolism observed in incubations with human liver microsomes was due to the cytochrome P450 3A4 enzyme, which produced the metabolites *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene, 9(10)-hydroxy-4-nitropyrene and 4-aminopyrene. An adduct that is probably responsible for the mutagenicity and carcinogenicity of 4-nitropyrene is *N*-(deoxyguanosin-8-yl)-4-aminopyrene. 4-Nitropyrene was mutagenic in bacteria, and at the thymidine kinase +/– locus in human lymphoblastoid cells. It did not cause cell transformation in cultured rat tracheal cells. No data were available on the cytogenetic effects, induction of mutation in oncogenes or tumour-suppressor genes, or on the effects on gene expression of 4-nitropyrene.

Overall, these results provide *moderate mechanistic evidence* to support the carcinogenicity of 4-nitropyrene.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 4-nitropyrene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-nitropyrene.

6.3 Overall evaluation

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

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