

**NTP REPORT ON CARCINOGENS BACKGROUND
DOCUMENT for 1-NITROPYRENE**

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NTP Report on Carcinogens Listing for 1-Nitropyrene

Carcinogenicity

1-Nitropyrene is *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in multiple species of experimental animals, at multiple sites and by multiple routes of exposure (reviewed in IARC V.46, 1989).

Intraperitoneal (i.p.) injections of 1-nitropyrene in the strain A mouse increased lung tumors in males and females, with the numbers of adenomas per mouse lung also increased (El-Bayoumy and Hecht, 1983; El-Bayoumy et al., 1984). When administered by subcutaneous (s.c.) injections, 1-nitropyrene induced injection site sarcomas in male and female rats and mammary tumors (including adenocarcinomas) in female rats (Hirose et al., 1984; cited by IARC V.46, 1989; Imaida et al., 1995). Intraperitoneal injections of 1-nitropyrene in mice caused liver-cell tumors in males (Wislocki et al., 1986; cited by IARC V.46, 1989). A study in female rats injected i.p. with 1-nitropyrene showed increased mammary tumors; a second i.p. study demonstrated a nonstatistically significant increase in mammary tumors (IARC V.46, 1989; Imaida et al., 1991a). Mammary gland tumors were also increased following oral administration of 1-nitropyrene to female rats (El-Bayoumy et al., 1988; 1995). Intratracheal administration of 1-nitropyrene, adsorbed onto carbon black particles, to hamsters demonstrated a weak but significant increase in lung tumors over particle only controls (Moon, et. al., 1990).

There are no adequate data available to evaluate the carcinogenicity of 1-nitropyrene in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

1-Nitropyrene has been evaluated for carcinogenicity in several other rodent studies by various routes of exposure with generally negative results; however, these studies are limited by small numbers of experimental animals and short durations of dosing and observation (IARC V.46, 1989). 1-Nitropyrene is genotoxic in a wide variety of assays in bacteria and mammalian cells including human cells and cells from likely target organs i.e. lung, demonstrates consistent evidence of cell transformation activity *in vitro* in both finite life-span and immortal cell lines including human cells, and has demonstrated ability to form DNA adducts *in vitro* and *in vivo*. Importantly, adducts have been detected in the lung following intratracheal instillation of 1-nitropyrene thus supporting potential genotoxic activity in a likely target organ in humans (IARC V.46, 1989; Chan, 1996).

No data are available that would suggest that the mechanisms thought to account for tumor induction of 1-nitropyrene in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

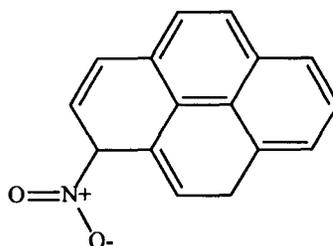
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

1-Nitropyrene [5522-43-0]



1.1 Chemical Identification

1-Nitropyrene (C₁₆H₉NO₂, mol. wt. = 247.25) is also called:

Pyrene, 1-nitro- (8CI, 9CI)
3-Nitropyrene
1-NP

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Yellow	Prager and Jacobson (1922; cited by IARC, 1989)
Physical State (Room Temp.)	Solid (Needle or prism crystals)	Prager and Jacobson (1922; cited by IARC, 1989)
Melting Point (C)	155	Luckenbach (1980; cited by IARC, 1989)
Solubility		
In water	< 0.1 g/100mL at 18 C	ChemFinder (1998)
Organic solvents:	Very soluble in diethyl ether Soluble in ethanol, benzene, toluene, and tetrahydrofluorenone	Prager and Jacobson (1992) Luckenbach (1980); Chemsyn Science Laboratories (1988); both cited by IARC (1989)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

- N*-Acetyl-1-aminopyrene (1-Acetylaminopyrene, 1-Acetamidopyrene, (C₁₈H₁₃NO, CASRN 22755-15-3, MW = 259.31)
- 1-Amino-3-hydroxypyrene (C₁₆H₁₂NO, CASRN 103114-40-5, MW = 234.28)
- 1-Amino-6-hydroxypyrene (C₁₆H₁₂NO, CASRN 1732-30-5, MW = 234.28)
- 1-Amino-8-hydroxypyrene (C₁₆H₉NO₃, CASRN 1732-31-6, MW = 234.28)
- 1-Aminopyrene (1-AP, C₁₆H₁₁N, CASRN 1606-67-3, MW = 217.27)
- 4,5-Epoxy-4,5-dihdropyrene (C₁₆H₁₀O₁, MW = 218.25)
- 3-Hydroxy-*N*-acetyl-1-aminopyrene (C₁₈H₁₄NO₂, MW = 276.31)
- 6-Hydroxy-*N*-acetyl-1-aminopyrene (C₁₈H₁₄NO₂, MW = 276.31)
- 8-Hydroxy-*N*-acetyl-1-aminopyrene (C₁₈H₁₄NO₂, MW = 276.31)
- N*-Hydroxy-1-aminopyrene (1-Hydroxylaminopyrene, C₁₆H₁₁NO, MW = 233.27)
- 3-Hydroxy-1-nitropyrene (1-Nitropyren-3-ol, C₁₆H₉NO₃, CASRN 86674-49-9, MW = 263.25)
- 6-Hydroxy-1-nitropyrene (1-Nitropyren-6-ol, C₁₆H₉NO₃, CASRN 1767-28-8, MW = 263.25)
- 8-Hydroxy-1-nitropyrene (1-Nitropyren-8-ol, C₁₆H₁₂NO₃, CASRN 1732-29-2, MW = 263.25)
- 10-Hydroxy-1-nitropyrene (1-Nitropyren-10-ol; 10-OH-1-NP, C₁₆H₉NO₃, CASRN 91254-92-1, MW = 263.25)
- 1-Nitro-5-H-phenanthro[4,5,6-*bcd*]pyran-5-one
- 1-Nitropyrene-*trans*-4,5-dihydrodiol (C₁₆H₁₁NO₄, CASRN 86674-50-2, MW = 281.27)
- 1-Nitropyrene-*trans*-9,10-dihydrodiol (C₁₆H₁₁NO₄, MW = 281.27)
- 1-Nitropyrene-4,5-oxide (1-NP-4,5-epoxide, C₁₆H₉NO₃, CASRN 102822-04-8, MW = 261.24)
- 1-Nitropyrene-9,10-oxide (1-NP-9,10-epoxide, C₁₆H₉NO₃, CASRN 105596-43-8, MW = 261.24)
- 1-Nitrosopyrene (C₁₆H₉NO, CASRN 86674-51-3, MW = 231.25)
- 1-Pyrenol (1-Hydroxypyrene; Pyren-1-ol, C₁₆H₁₀O, CASRN 78751-40-3, MW = 218.25)
- Pyrenylnitrenium ion (Nitrenium ion, C₁₆H₁₀N, MW = 216.26)

Physical-chemical properties were found for only 1-aminopyrene. Structures for at least some of these analogues may be found in Figures 6-1, 6-2, and 6-3.

1-Aminopyrene

Property	Information	Reference
Solubility		
Organic solvents	Soluble in ethyl ether and benzene	Weast and Astle (1980)

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Use

1-Nitropyrene (1-NP) has been reported to be a chemical photosensitizer, increasing the spectral sensitivity of bis-azide compounds in the long-wavelength region. One foreign company uses 1-NP as an intermediate in the production of 1-azidopyrene, which is used in photosensitive printing. 1-NP is available for research purposes at 97% or 99.5% purity with 0.1% total dinitropyrenes and pyrene. It is available at a purity of 99.68% as a reference material (IARC, 1989).

2.2 Production

Since 1972, one foreign company has produced 1-NP by the reaction of pyrene with nitric acid (IARC, 1989). One U.S. company produces 1-NP (SRI, 1992). No data on imports or exports of 1-NP were available. Chem Sources (1996) identified 11 U.S. suppliers of 1-NP.

2.3 Exposure

2.3.1 Environmental Exposure

The primary route of potential human exposure to 1-NP is inhalation. 1-NP is one of the most abundant ambient air mononitroarenes. Low concentrations of 1-NP have been found in ambient airborne particulates. 1-NP has also been detected in stack gases from coal-fired power plants and aluminum smelters, and in particulate emissions from other stationary sources and from diesel and gasoline engines. Prior to 1980, some carbon black samples known to be used in photocopy machines were found to contain considerable quantities of nitropyrenes. 1-NP has also been detected in the wastewater from gasoline service stations and in river sediment (IARC, 1989).

2.3.1.1 Ambient and Residential Air

Nitro-polycyclic aromatic hydrocarbons (NPAH) formed from atmospheric reactions of polycyclic aromatic hydrocarbons (PAH) with nitrogen oxides have been detected in urban air (Pitts et al., 1978; Gibson et al., 1983; both cited by Heitkamp et al., 1991). 1-NP was detected in ambient air samples collected in Torrance, California, during a 6-year study that evaluated the gas-phase atmospheric reactions of PAH and their nitroderivatives. The authors attributed its presence in ambient air samples to direct emission from combustion sources (Zielinska et al., 1990). In Houston, Texas, the concentration of 1-NP in vapor plus particulate phase in ambient air was 9.3 pg/m³ when measured over a 12-month period by GC-MS (Wilson et al., 1995).

In the environment, 1-NP is photodegraded to the major product 9-hydroxy-1-nitropyrene, a less mutagenic compound (Saito et al., 1993; Koizumi et al., 1994). The concentration of 9-hydroxy-1-nitropyrene in urban air was 15 to 23 pg/m³ (Saito et al., 1993).

The indoor (kitchen mean, 0.044 $\mu\text{g}/\text{m}^3$ and living room, 0.049 $\mu\text{g}/\text{m}^3$) and mean outdoor concentrations (0.023 $\mu\text{g}/\text{m}^3$) of 1-NP were determined in an 8-home study in Columbus, Ohio. The 8 homes were chosen on the basis of several characteristics: electric/gas heating system, electric/gas cooking appliances, and the absence/presence of environmental tobacco smoke (Chuang et al., 1991).

The use of unvented kerosene space heaters resulted in an increase of 1-NP in airborne particulate (Traynor et al., 1990; Mumford et al., 1991). Air samples collected from a home in Apex, North Carolina, that had a kerosene heater in the living room turned off, showed an average concentration of 0.081 ng/m^3 1-NP for samples collected in the first week of a 4-week sampling period, while samples collected in the same house with the kerosene heater on during the fifth week following the 4-week sampling period showed an average concentration of 0.057 ng/m^3 by GC-MS (Mumford et al., 1991).

2.3.1.2 Vehicular Emissions (see also 2.3.2 Occupational Exposure)

1-NP and other nitroarenes have been detected exhaust particulates from idling gasoline and diesel engines. Total concentrations from diesel engine vehicles are significantly higher than those from gasoline engine vehicles (e.g., Hayakawa et al., 1994).

2.3.1.3 Water

1-NP biodegraded slowly in river water that had a low number of colony-forming units (CFU); in contrast, 1-NP biodegraded rapidly in aerobic soil suspensions containing a high number of CFU, suggesting that environmental microflora are important for primary degradation of relatively low concentrations of nitropyrenes (Tahara et al., 1995).

2.3.1.4 Other Media

Relatively high concentrations of 1-NP were found in grilled chicken but not in grilled and smoked sausage and fish (Ishinishi et al., 1986, cited by Scheepers et al., 1994b). It has also been found in cigarette smoke condensate (Tokiwa and Ohnishi, 1986) and methanolic extracts of fly ash from coal combustion (Wilson et al., 1984).

2.3.2 Occupational Exposure

1-NP has been found in airborne particulate matter from several workplace atmospheres contaminated with diesel exhaust: River vessel (workplace), ship's engine (source of diesel exhaust); repair shop for trains, train engines; Army driving lessons, armored cars; flower auction, trucks; farming, tractor; gardening, passing traffic; airport platform, platform vehicles; concrete manufacturing, chemical plant, aluminum rolling, and galvanizing workshop, fork-lift trucks; grass verge maintenance, lawn mowers; and a river vessel, ships aggregate (Scheepers et al., 1994a, 1994b, 1995).

1-NP was not listed in the National Occupational Exposure Survey (1984) or the National Occupational Hazard Survey (1986) conducted by NIOSH.

2.4 Regulations

1-NP is subject to report/record keeping requirements under SARA. OSHA regulates 1-NP under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulation	Summary of Regulation
E P A	40 CFR 372. Promulgated 2/16/88. Amended 1/12/94. SARA 313: Proposed rule to add 313 chemicals and chemical categories (including 1-NP as a polycyclic aromatic compound) to the list of toxic chemicals required to be reported under section 313 of the Emergency Planning and Community Right-to-Know ACT.	Would require public notice of the release of a toxic chemical and also require suppliers to notify persons to whom they distribute of the presence of these chemicals in their products. Comments on this proposed rule were to have been received by 4/12/94.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of 1-NP in humans.

4.0 MAMMALIAN CARCINOGENICITY

Full details of mammalian carcinogenicity studies of 1-NP are presented in Table 4-1.

4.1 Mice**4.1.1 Subcutaneous Injection**

Treatment once per week by subcutaneous (s.c.) injection of 6-week-old male BALB/c mice with 0.1 mg (0.4 μmol) 1-NP in 0.2 mL DMSO for 20 weeks did not significantly increase the incidence of injection site tumors or lung tumors. All mice were observed for 60 weeks or until moribund (Tokiwa et al., 1984; cited by IARC, 1989).

4.1.2 Intraperitoneal Injection

Newborn male CD-1 mice treated with a 2.8 μmol total dose of 1-NP (administered as three injections; on days 1, 8, and 15 after birth) had a significantly increased incidence of liver tumors (adenoma and carcinoma) compared to vehicle controls. Females treated with this total dose (2.8 μmol) and males and females treated with a lower total dose (0.7 μmol) did not have a significantly increased incidence of liver tumors. Males and females did not have a significantly increased incidence of lung tumors or malignant lymphomas. All mice were observed for 1 year or until moribund (Wislocki et al., 1986; cited by IARC, 1989).

There was no significant increase in the incidence or multiplicity of lung adenoma or adenocarcinoma in newborn Swiss-Webster BLU:Ha mice treated with a total dose of 21 or 105 μg (0.085 or 0.42 μmol) 1-NP over 15 days (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15) as compared to controls killed at 26 weeks of age (Busby et al., 1989).

Negative results were also found for newborn B6C3F1 mice treated with a total dose of 0.7 μmol 1-NP over 15 days (1/7 of total dose [0.1 μmol] administered at birth, 2/7 [0.2 μmol] on day 8, 4/7 [0.4 μmol] on day 15). No significant increase in the tumor incidence in 1-NP-treated mice as compared to vehicle or shelf controls was found when mice were killed at 72 weeks of age (Mori et al., 1992).

Six- to 8-week-old male and female A/J mice treated with 3 injections of 1-NP per week for 6 weeks (6440 $\mu\text{mol/kg}$ total dose) and sacrificed 18 weeks after the start of treatment may have had a significantly increased incidence of lung tumors, but an inappropriate statistical test (Student's *t*-test) was used to analyze the significance of tumor incidence. Males treated with a lower total dose (710 $\mu\text{mol/kg}$) also had an increased incidence of lung tumors. However, males treated with an intermediate total dose (2140 $\mu\text{mol/kg}$) did not have a significantly increased incidence of lung tumors. Females treated with either of the two lower total doses (710 or 2140 $\mu\text{mol/kg}$) did not have a significantly increased incidence of lung tumors. The multiplicity of lung tumors was significantly increased in high-dose mice, but not in low- or mid-dose mice (El-Bayoumy et al., 1984).

4.2 Rats

4.2.1 Oral Administration

The incidence of mammary tumors was significantly increased in 30-day-old female CD rats treated with 50 μmol 1-NP, once per week for 8 weeks and sacrificed after 41 weeks (El-Bayoumy et al., 1995), in newborn female SD rats treated with 100 or 250 $\mu\text{mol/kg}$ mean body weight for 16 weeks and sacrificed after 94 weeks (El-Bayoumy et al., 1988a), and in 6-week-old female specific-pathogen-free F344/JcI rats treated with 10 or 20 mg 1-NP/kg mean body weight (40 or 81 $\mu\text{mol/kg}$; 1-NP contaminated with dinitropyrenes), twice per week for 55 weeks and sacrificed after 104 weeks (Odagiri et al., 1986; cited by IARC, 1989). Weanling female CD rats treated with 10 μmol 1-NP/kg mean body weight, 3 times per week for 4 weeks, and sacrificed after 76 to 78 weeks however, did not have a significantly increased incidence of mammary tumors compared to vehicle controls (King, 1988).

The incidence of clitoral gland tumors and the incidence of mononuclear-cell leukemia were significantly increased in 6-week-old female specific-pathogen-free F344/JcI rats treated with 5 to 20 mg 1-NP/kg mean body weight (20 to 81 $\mu\text{mol/kg}$), twice per week for 55 weeks (Odagiri et al., 1986; cited by IARC, 1989).

4.2.2 Subcutaneous Injection

The incidence of mammary tumors was significantly increased in weanling female CD rats treated with 100 μmol 1-NP/kg mean body weight, once per week for 5 weeks, and sacrificed after 88 weeks (King, 1988; Imaida et al., 1991a); in newborn female CD rats treated with a 6.3 μmol total dose of 1-NP over an 8-week period (week 1: 2.5 $\mu\text{mol/kg/wk}$, weeks 2-3: 5 $\mu\text{mol/kg/wk}$, weeks 4-8: 10 $\mu\text{mol/kg/wk}$) and sacrificed after 67 weeks (King, 1988; Imaida et al., 1995), and in newborn female CD (but not F344) rats treated with 100 μmol 1-NP/kg mean body weight, once per week for 8 weeks, and sacrificed after 78 weeks (Imaida et al., 1995; King, 1988; Hirose et al., 1984; cited by IARC, 1989).

The incidence of leukemia was significantly increased in newborn female F344 (but not CD) rats treated with 100 μmol 1-NP/kg mean body weight, once per week for 8 weeks, and sacrificed after 78 weeks (King, 1988; Imaida et al., 1995).

The incidence of injection-site tumors was significantly increased in newborn male and female CD rats treated with 100 μmol 1-NP/kg mean body weight, once per week for 8 weeks, and sacrificed at 62 weeks of age (Hirose et al., 1984; cited by IARC, 1989) and in 8-week-old male F344/DuCrj rats treated with 2 mg (8 μmol) 1-NP (possibly contaminated with 0.8%

dinitropyrenes), twice per week for 10 weeks, and sacrificed after up to 377 days (Ohgaki et al., 1982).

No injection-site tumors were detected in 6-week-old Fischer F344/DuCrj rats treated with 0.2 or 2 mg (0.8 or 8 μmol) 1-NP (impurities: < 0.05% each of 1,3-, 1,6-, and 1,8-dinitropyrene, 1,3,6-trinitropyrene, and 1,3,6,8-tetranitropyrene) in 0.2 mL DMSO twice per week for 10 weeks and sacrificed after 650 days (Ohgaki et al., 1985; cited by IARC, 1989).

4.2.3 Intraperitoneal Injection

The incidence of mammary tumors was significantly increased in weanling female CD rats treated with 10 μmol 1-NP/kg mean body weight, 3 times per week for 4 weeks, and sacrificed after 76 to 78 weeks (King, 1988), and in weanling female CD rats treated with 100 μmol 1-NP/kg mean body weight, once per week for 4 weeks, and sacrificed after 87 to 90 weeks (King, 1988; Imaida et al., 1991a).

In 30-day-old female CD rats treated with 67 μmol 1-NP/kg mean body weight 3 times per week for 4 weeks and sacrificed after 61 weeks, there was no significant increase in tumor incidence (Imaida et al., 1991a).

4.2.4 Intrapulmonary Administration

Injection of 1.5 mg (6.1 μmol) 1-NP in 0.05 mL beeswax tricapyrylin, after left lateral thoracotomy, into the lower third of the left lung of 10- to 11-week-old male F344/DuCrj rats did not produce squamous-cell carcinoma of the lung in any treated animals that were observed for 72 weeks (Maeda et al., 1986; cited by IARC, 1989).

4.2.5 Mammary Injection

Injection of a total dose of 12.3 μmol 1-NP into the thoracic and inguinal nipples of 30-day-old female CD rats did not increase the incidence or multiplicity of mammary tumors in treated animals after 77 weeks (Imaida et al., 1991a).

4.2.6 Inhalation Exposure

Nose-only exposure of 10- to 11-week old male and female F344/CrI rats to aerosol containing 1-NP ($\sim 7 \text{ mg/m}^3$ [$\sim 30 \mu\text{mol/m}^3$]), alone or in combination with SO_2 and/or Ga_2O_3 , 2 hours per day for 4 weeks did not significantly increase the incidence of lung lesions, nasal lesions, or kidney tumors in animals exposed only to 1-NP and sacrificed after 1 day, 2 weeks, 2 months, 6 months, or 12 months (Wolff et al., 1989).

4.3 Hamsters

In a study using a route relevant to that of human exposure, lung albeit by instillation, treatment of 7- to 9-week-old male Syrian golden hamsters with 1 or 2 mg (4 or 8 μmol) 1-NP adsorbed onto an equal mass of carbon carrier particles, once or twice per week for 92 weeks demonstrated a weak dose response increase in lung tumors, though the increase was not statistically significant. Animals were sacrificed at the end of the treatment period (Moon et al., 1990). In another study that used contaminated 1-NP (0.008% 1,3-DNP, 0.6% 1,6- plus 1,8-DNP, and 1.3% pyrene), treatment of 8-week-old male Syrian golden hamsters with 2 mg (8 μmol) 1-NP, once per week for 15 weeks significantly increased the incidence of lung adenoma. Hamsters were observed for life (up to 663 days) (Yamamoto et al., 1987; cited by IARC, 1989).

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - Subcutaneous Injection							
6-wk-old BALB/c mice	20M	20M (DMSO alone)	1-NP, > 99.9% pure	0.1 mg (0.4 µmol) in 0.2 mL DMSO, s.c. injection, once/wk	20 wk	<p>All mice were observed for 60 weeks (it was not specified whether observation began at the beginning or at the end of treatment) or, for mice with injection-site tumors, until they were moribund. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Injection Site: Negative No s.c. injection-site tumors were detected in either 1-NP-treated or in control mice.</p> <p>Lung: Negative There was no significant increase in the incidence of lung tumors in 1-NP-treated mice.</p> <p>The statistical test used to analyze tumor incidence was not specified by IARC. IARC noted the small number of animals used and the short period of observation.</p>	Tokiwa et al. (1984; cited by IARC, 1989)
Mice - Intraperitoneal Injection							
newborn CD-1 mice	90 or 100 M and F per dose	<p>vehicle control group 1: 90 or 100 M and F</p> <p>vehicle control group 2 (began 10 wk after the first group): 90 or 100 M and F</p> <p>Positive controls (560 nmol B[a]P total dose): 90 or 100 M and F</p>	1-NP, > 99% pure	<p>0.7 or 2.8 µmol total dose in 10, 20, and 40 µL DMSO.</p> <p>Administered as 3 i.p. injections: on days 1, 8, and 15 days after birth.</p>	15 days	<p>Surviving mice were killed after 1 year. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Liver: Positive (for tumorigenesis in high-dose males) 1-NP did not induce liver tumors in females. Males in the high-dose group had a significantly increased incidence of tumors (8/29 [3 adenomas, 5 carcinomas] vs. 2/28 in control group 1 and 5/45 in control group 2). The incidence of liver tumors in males in the low-dose group was not significantly different from that in appropriate controls.</p> <p>Lung and Lymphatic System: Negative The incidence of lung tumors and malignant lymphomas in 1-NP-treated mice was not significantly different from that in vehicle controls.</p> <p>IARC noted the short observation period.</p>	Wislocki et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
newborn Swiss-Webster BLU:Ha mice	29M, 23F	91M, 101F (DMSO alone)	1-NP, > 99.9% pure	Total dose: 21 or 105 µg (0.085 or 0.42 µmol), administered i.p. (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15)	15 days	Mice were killed at 26 weeks of age. Lungs and abnormal tissues were examined histologically. Statistical analyses of tumor incidence and multiplicity were performed using the method of Peto et al. (1980; cited by Busby et al., 1989), without correction for intercurrent mortality, and Student's <i>t</i> -test, respectively. Lung: Negative There was no significant increase in the incidence or multiplicity of lung adenoma or adenocarcinoma in 1-NP-treated mice as compared to controls.	Busby et al. (1989)
newborn B6C3F1 mice	21M, 20F	39M, 38F (no treatment) 28M, 27F (DMSO alone)	1-NP, 99.99% pure	Total dose: 0.7 µmol, administered i.p. (1/7 of total dose [0.1 µmol] administered at birth, 2/7 [0.2 µmol] on day 8, 4/7 [0.4 µmol] on day 15)	15 days	Mice were killed when they reached 72 weeks of age. A complete postmortem examination was performed. Liver/Misc: Negative There was no significant increase in the tumor incidence in 1-NP-treated mice as compared to vehicle or shelf controls. The method used for statistical analysis of tumor incidence was not specified.	Mori et al. (1992)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6 to 8-wk-old A/J mice	15M, 14F (low dose) 15M, 14F (mid dose) 16M, 12F (high dose)	16M, 16F (trioctanoin alone)	1-NP, purity not specified	710 µmol/kg, 2140 µmol/kg, or 6440 µmol/kg, total dose in trioctanoin, administered as 17 i.p. injections (3/wk)	6 wk	<p>Mice were killed 18 weeks after treatment was completed. Only lungs were examined.</p> <p>The lung tumor response (percentage of mice that developed lung tumors and the number of lung tumors per mouse) in 1-NP-treated and control groups was compared by Student's <i>t</i>-test. [Student's <i>t</i>-test is not an appropriate statistical test for analyzing the significance of tumor incidence.]</p> <p>Lung: Positive (for tumorigenesis in high-dose males and females)</p> <p>Combined (male and female) lung tumor incidence was significantly increased in high-dose mice (14/16 males vs. 3/16 male controls; 8/12 females vs. 4/16 female controls; <i>p</i>-value not given), but not in low- or mid-dose mice.</p> <p>Low-dose males had an increased incidence of lung tumors, but mid-dose males did not. Low- and mid-dose females did not have an increased incidence of lung tumors.</p> <p>The multiplicity of lung tumors was significantly increased in high-dose mice (combined male and female group; 1.3-1.0 mean number lung tumors/animal vs. 0.3-0.6 tumors/control; <i>p</i> < 0.001), but not in low- or mid-dose mice.</p>	El-Bayoumy et al. (1984)
Rats - Oral Administration							
weanling CD rats	36F	36F (DMSO alone)	1-NP, > 99.9% pure	10 µmol/kg mean body weight in DMSO (1.7 µmol/mL), 3 times/wk, by intragastric intubation	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>All Tissues: Negative</p> <p>There was no significant increase in the incidence of tumors in 1-NP-treated rats as compared to controls.</p>	King (1988)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
30-day-old CD rats	30F	30F (trioctanoin alone) 30F (non-gavaged diet controls; sentinels)	1-NP, > 99.9% pure	50 µmol in 0.5 mL trioctanoin, once/wk by gavage	8 wk	<p>Rats were killed 41 weeks after last treatment. All organs and mammary glands were examined histologically.</p> <p>Mammary Gland: Positive (for fibroadenoma)</p> <p>Mammary fibroadenoma was detected in 10/30 1-NP-treated rats vs. 0/30 controls (p < 0.01, log-rank test).</p> <p>The multiplicity of fibroadenomas was significantly increased in 1-NP-treated rats (15 tumors/rat vs. none in controls; p < 0.05, Tukey's method).</p> <p>The incidences of mammary desmoplastic adenoma, adenoma, and adenocarcinoma were not significantly increased in 1-NP-treated animals.</p>	El-Bayoumy et al. (1995)
newborn SD rats	25M, 33F (low dose) 36M, 24F (high dose)	22M, 31F (trioctanoin alone)	1-NP, > 99.9% pure	<p>100 µmol/kg mean body weight in trioctanoin (16.2 µmol/mL trioctanoin), once/wk by gavage</p> <p>250 µmol/kg mean body weight in trioctanoin (40.5 µmol/mL trioctanoin), once/wk by gavage</p>	16 wk	<p>Animals were observed for 94 weeks. All organs and gross lesions were examined histologically. Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Mammary Gland: Positive (for adenocarcinoma; females only)</p> <p>The incidence of adenocarcinoma in females treated with either the low or high dose was significantly increased (14/33 and 15/24 vs. 1/31 controls; p < 0.01).</p> <p>The incidence of adenoma was not significantly increased in 1-NP-treated rats.</p> <p>Other: There was an increase in the incidence of lung tumors in high-dose females (3/24 vs. 0/31 controls; p-value not given) and of pancreatic tumors in low-dose males (4/25 vs. 0/22 controls; p-value not given).</p> <p>It was also reported that 1-NP was significantly more carcinogenic than its metabolites 1-nitrosopyrene or 1-aminopyrene.</p>	El-Bayoumy et al. (1988a)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old specific-pathogen-free F344/Jcl rats	40F (low dose) 40F (mid dose) 46F (high dose)	30F (olive oil alone)	1-NP, containing 0.11% 1,3-DNP, 0.27% 1,6-DNP, 0.23% 1,8-DNP	5, 10, or 20 mg/kg mean body weight (20-81 µmol/kg mean body weight), twice/wk, by intragastric installation	55 wk	<p>Rats were killed when moribund or after 104 weeks. Only those rats surviving beyond experimental week 46 were evaluated. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Mammary Gland: Positive (for carcinogenesis with mid and high dose)</p> <p>Adenocarcinomas were detected in 2/36 (not significant), 12/39, and 14/45 low-dose, mid-dose, and high-dose rats, respectively, vs. none in vehicle controls.</p> <p>Clitoral gland: Positive (for tumorigenesis)</p> <p>Clitoral gland tumors (mainly squamous cell carcinomas) were detected in 11/39 mid-dose rats (9 with squamous cell carcinomas) and in 12/45 high-dose animals (11 with squamous cell carcinomas) vs. 1 adenoma in 30 controls. The incidence, although not specified, of clitoral gland tumors in low-dose rats was not significantly different from that in controls.</p> <p>Blood: Positive (for mononuclear-cell leukemia)</p> <p>Mononuclear-cell leukemia was detected in 23/36 low-dose, 22/39 mid-dose, and 27/45 high-dose rats vs. 9/28 controls.</p> <p>IARC noted the presence of DNP contaminants and that their effect on the results could not be determined.</p>	Odagiri et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Subcutaneous Injection							
weanling CD rats	29F	30F (DMSO alone)	1-NP, > 99.9% pure	100 µmol/kg mean body weight in 70 µmol DMSO/mL, once/wk, injected s.c.	5 wk	<p>Rats were observed until moribund or up to 88 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Mammary Gland: Positive (for fibroadenoma)</p> <p>The incidence of fibroadenoma was significantly increased in 1-NP-treated rats (15/29 vs. 8/30 controls, $p < 0.04$). The mean induction time for mammary tumors was greater than 70 weeks for both 1-NP-treated and control rats and did not differ significantly between these two groups.</p>	King (1988) Imaida et al. (1991a)
newborn CD rats	49F	40F (DMSO alone)	1-NP, > 99.9% pure	<p><i>week 1:</i> 2.5 µmol/kg mean body weight/wk</p> <p><i>weeks 2-3:</i> 5 µmol/kg mean body weight/wk</p> <p><i>weeks 4-8:</i> 10 µmol/kg mean body weight/wk</p> <p>6.3 µmol average total dose/rat, injected s.c.</p>	8 wk	<p>Rats were observed until moribund or up to 67 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Mammary Gland: Positive (for adenocarcinoma)</p> <p>The incidence of adenocarcinoma was significantly increased in 1-NP-treated animals (10/49 vs. 1/40 controls, $p < 0.025$). The mean induction time for mammary tumors did not differ significantly between 1-NP-treated and control groups.</p>	King (1988) Imaida et al. (1995)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
newborn CD rats	48F (CD rats)	47F (CD rats;	1-NP, >99.9% pure	100 µmol/kg mean body weight in DMSO (70 µmol/mL DMSO), once/wk, injected s.c. total average dose = 63 µmol/CD rat	8 wk	Rats were killed when moribund or after 78 weeks of observation. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically. Statistical analysis of tumor incidence was performed using the χ^2 test.	King (1988)
newborn F344 rats	55F (F344 rats)	55F (F344 rats; DMSO alone)	1-NP, >99.9% pure	100 µmol/kg mean body weight in DMSO (70 µmol/mL DMSO), once/wk, injected s.c. total average dose = 40 µmol/F344 rat	8 wk	Mammary Gland: Positive (for adenocarcinoma; CD rats only) The incidence of adenocarcinoma was significantly increased in CD rats (10/48 vs. 3/47 controls; $p < 0.05$), but not in F344 rats. The mean induction time for mammary tumors did not differ between 1-NP-treated and control groups. Blood: Positive (for leukemia; F344 rats only) The incidence of leukemia was significantly increased in F344 rats (4/55 vs. 0/55 controls; $p < 0.05$), but not in CD rats.	Imaida et al. (1995)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
newborn SD-derived CD rats	29M, 31F (low dose) 31M, 32F (high dose)	28M, 31F (DMSO alone)	1-NP, containing < 0.02% DNPs	50 or 100 µmol/kg mean body weight, once/wk, injected s.c.	8 wk	<p>The experiment was terminated when rats were 62 weeks old. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Injection site: Positive (for tumorigenesis with high dose)</p> <p>In the high-dose group, 10/31 males and 9/32 females developed sarcomas (mainly malignant fibrous histiocytomas) vs. none of the controls. The incidence of sarcoma in low-dose animals was not significantly different from the control incidence.</p> <p>Mammary Gland: Positive (for tumorigenesis with high dose)</p> <p>In the high-dose group, 15/32 females (vs. 2/31 controls) developed mammary tumors (10 adenocarcinomas, 7 fibroadenomas). The incidence of mammary tumors in the low-dose group was not significantly different from the incidence in controls.</p> <p>Other Tissues: Negative</p> <p>Tumors were observed in some other tissues (not specified) of treated rats, but their incidences were not significantly different from controls.</p>	Hirose et al. (1984; cited by IARC, 1989)
6-wk-old F344/DuCrj rats	10M (low dose) 20M (high dose)	20M (DMSO alone)	1-NP, containing < 0.05% each of 1,3-, 1,6-, and 1,8-DNP, 1,3,6-trinitropyrene, and 1,3,6,8-tetranitropyrene	0.2 or 2 mg (0.8 or 8 µmol) in 0.2 mL DMSO, 2 times/wk, injected s.c.	10 wk	<p>Animals were observed for 650 days.</p> <p>Injection Site: Negative</p> <p>No injection-site tumors were detected in treated rats or in controls.</p> <p>IARC noted the small number of animals used and the short observation period.</p>	Ohgaki et al. (1985; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
8-wk-old F344/DuCrj rats	20M	20M (DMSO alone)	1-NP, >99% pure (see last entry in Results/Comments)	2 mg (8 µmol) in 0.2 mL DMSO, 2 times/wk, injected s.c.	10 wk	<p>All rats were observed for life (the last rat died on day 377). All organs were examined histologically. The first tumor in 1-NP-treated rats was detected on day 162.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Injection Site: Positive (for malignant fibrous histiocytoma)</p> <p>Seven of 17 1-NP-treated rats (vs. none of controls) surviving beyond 162 days had malignant fibrous histiocytoma at the injection site. The statistical significance of this was not specified.</p> <p>Bone: One of 17 1-NP-treated rats (vs. none of the controls) surviving beyond 162 days had an extraskeletal osteosarcoma. The significance of this tumor was not specified.</p> <p>The combined incidence of malignant fibrous histiocytoma and osteosarcoma was significantly increased in 1-NP-treated rats (8/17 vs. none in controls; $p < 0.003$).</p> <p>IARC (1989) noted that in a later study (Ohgaki et al., 1985; cited by IARC, 1989), the authors reported that the 1-NP used in this study was possibly contaminated with about 0.8% DNPs.</p>	Ohgaki et al. (1982)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Intraperitoneal Injection							
weanling CD rats	36F	31F (DMSO alone)	1-NP, > 99.9% pure	10 µmol/kg mean body weight in DMSO (1.7 µmol/mL DMSO), 3 times/wk, injected i.p.	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Mammary Gland: Positive (for tumorigenesis)</p> <p>The incidences of adenocarcinoma and fibroadenoma were significantly increased in 1-NP-treated animals (adenocarcinoma: 14/36 vs. 3/31 controls, $p < 0.01$; fibroadenoma: 19/36 vs. 5/31 controls, $p < 0.001$). The average induction period for mammary tumors, however, did not differ significantly between 1-NP-treated and control groups.</p>	King (1988)
30-day-old CD rats	29F	29F (DMSO alone)	1-NP, purity not specified	67 µmol/kg mean body weight, in DMSO (25 µmol/mL DMSO) 3 times/wk, injected i.p.	4 wk	<p>Rats were killed 61 weeks after the first injection. All organs were examined macroscopically and histologically; particular attention was given to the mammary glands.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>All Organs: Negative</p> <p>The incidence of tumors was not significantly increased in 1-NP-treated rats.</p>	Imaida et al. (1991a)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
weanling CD rats	29F	30F (DMSO alone)	1-NP, > 99.9% pure	100 µmol/kg mean body weight in DMSO (70 µmol/mL DMSO), once/wk, injected i.p.	5 wk	<p>Rats were observed until moribund or up to 88 weeks. All organs were examined macroscopically. The brain, pituitary gland, mammary glands, thyroid gland, esophagus, bronchus, lungs, stomach, small intestine, large intestine, liver, kidneys, spleen, ovaries, preputial gland, and any pathological lesions observed macroscopically were examined histologically.</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>Mammary Gland: Positive (for adenocarcinoma)</p> <p>The incidence of adenocarcinoma was significantly increased in 1-NP-treated rats (8/29 vs. 4/30 controls, $p < 0.03$). The mean induction time for mammary tumors was greater than 70 weeks for both 1-NP-treated and control rats and did not differ significantly between these two groups.</p>	King (1988) Imaida et al. (1991a)
Rats - Intrapulmonary Administration							
10- to 11-wk-old F344/DuCrj rats	32M	31M (beeswax-tricaprylin alone)	1-NP, > 99.9% pure	1.5 mg (6.1 µmol) in 0.05 mL beeswax-tricaprylin, single dose injected into the lower third of left lung after left lateral thoracotomy	single dose	<p>Rats were observed for 72 weeks after treatment. IARC did not specify which organs were examined.</p> <p>Lung: Negative</p> <p>Squamous-cell carcinoma was not detected in 1-NP-treated or control rats.</p> <p>IARC noted the short observation period.</p>	Maeda et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Mammary Injection							
30-day-old CD rats	27F	DMSO alone; 28F	1-NP, purity not specified	<p><i>day 1:</i> 0.1 mL of 20.3 µmol/mL DMSO injected directly into each of 3 left thoracic nipples.</p> <p>Right thoracic nipples injected with DMSO alone.</p> <p><i>Day 2:</i> 0.1 mL of 20.3 µmol/mL DMSO injected directly into left inguinal nipples.</p> <p>Right inguinal nipples injected with DMSO alone.</p> <p>Total dose: 12.3 µmol/animal</p>	single injection	<p>Surviving rats were killed at 77 weeks. All organs were examined macroscopically and histologically; "particular attention was given to the mammary glands."</p> <p>Statistical analysis of tumor incidence was performed using the χ^2 test.</p> <p>All Organs: Negative</p> <p>The incidence of tumors was not significantly increased in 1-NP-treated rats.</p>	Imaida et al. (1991a)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Inhalation Exposure							
10- to 11-wk-old F344/Crl rats	40M, 40F	40M, 40F (air alone)	1-NP, > 99.9% pure	<p>Animals were exposed nose-only to aerosol containing 1-NP, alone or in combination with SO₂ and/or Ga₂O₃, 2 h/day, 5 days/wk</p> <p>6.6 mg 1-NP/m³ (27 μmol/m³)</p> <p>6.5 mg 1-NP/m³ (26 μmol/m³) + 5.0 ppm SO₂</p> <p>7.3 mg 1-NP/m³ (29 μmol/m³) + 26.2 mg Ga₂O₃/m³</p> <p>7.4 mg 1-NP/m³ (30 μmol/m³) + 25.8 mg Ga₂O₃/m³ + 5.0 ppm SO₂</p>	4 wk	<p>Groups of 3 males and 3 females were killed for histopathological evaluations 1 day, 2 weeks, 2 months, 6 months, and 12 months after the 4-week exposure period. Histopathological examinations were performed on lung, nasal sections, and kidney. The statistical test used to analyze tumor incidence was not specified.</p> <p>Lung: Negative</p> <p>In rats exposed to Ga₂O₃, lung lesions were non-neoplastic. Significant lung lesions were not observed in any treated rats that were not exposed to Ga₂O₃.</p> <p>Nasal Cavity: Negative</p> <p>Significant nasal lesions were not observed in any of the rats.</p> <p>Kidney: Negative</p> <p>No kidney tumors were reported. Rats killed at 6 months and 1 year displayed nephropathy, but it was dismissed as a common trait in aged rats.</p>	Wolff et al. (1989a)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Hamsters - Intratracheal Instillation							
8-wk-old Syrian golden hamsters	34M	19M (phosphate buffer solution alone)	1-NP, 98% pure, containing 0.008% 1,3-DNP, 0.6% 1,6- plus 1,8-DNP, and 1.3% pyrene	2 mg (8 µmol) suspended in 0.2 mL phosphate buffer solution, once/wk, by intratracheal instillation	15 wk	<p>Hamsters were observed until death (1-NP-treated hamsters: up to 663 days after first treatment, controls: up to 684 days after first treatment). Three 1-NP-treated hamsters were cannibalized. [Group housing of hamsters is unusual.] The organs examined and the statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Lung: Positive (for tumorigenesis)</p> <p>Two lung adenomas were detected in 2/21 1-NP-treated hamsters. [Historically, hamsters are highly resistant to lung tumors.]</p> <p>Trachea: In one of the hamsters with lung adenoma, squamous-cell papilloma in the trachea was observed.</p> <p>1-NP was contaminated with dinitropyrenes and pyrenes.</p>	Yamamoto et al. (1987; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity Studies of 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
7- to 9-wk-old Syrian golden hamsters	55M (2mg [8.09 µmol], once/wk) 55M (1 mg [4.04 µmol], once/wk) 58M (2 mg [8.09 µmol], twice/wk) 60M (1 mg [4.04 µmol], twice/wk)	50M (sterile saline alone) 50M (carbon particles suspended in saline) 50M (B[a]P; positive control) 20M (no treatment; shelf control)	1-NP, 99.9% pure	1 or 2 mg (4 or 8 µmol) adsorbed onto an equal mass of carbon carrier particles (Stokes diameter 2-5 µm, greater than 70%), once or twice/wk, administered intratracheally	92 wk	<p>Hamsters that died or were found moribund during the treatment period were immediately necropsied. The study was terminated after 92 weeks of treatment, at which time all surviving hamsters were necropsied. The larynx, trachea, and other tissues showing gross abnormalities were examined.</p> <p>1-NP-treated hamsters displayed a dose-related decrease in survival and body-weight gain. In order to correct for a high incurrent mortality in the control groups, tumor incidences were evaluated after modeling the effect of treatment in all hamsters for the time they were alive. This was done via Cox regression. Analysis of tumor incidence was performed using a log-linear model. If the log-linear model indicated a significant effect, pairwise comparisons were made using the ² test.</p> <p>Trachea: Negative</p> <p>There was no significant difference in the incidence of neoplastic lesions (papilloma, polyps, carcinoma in situ, squamous cell carcinoma, anaplastic carcinoma, basal cell carcinoma, unspecified carcinoma) between treated hamsters and appropriate controls.</p> <p>Lung: Negative</p> <p>There was no significant difference in the incidence of neoplastic lesions (adenoma, mastocytoma, bronchial carcinoma, papillary carcinoma, adenocarcinoma, squamous cell carcinoma, mixed squamous adenomatous carcinoma, alveolar bronchiolar carcinoma, anaplastic carcinoma) between treated hamsters and appropriate controls.</p> <p>Lung and Trachea: The mean latency time for tumors was significantly lower when the high dose was administered twice/wk than when it was administered once/wk (p < 0.01; Mantel-Cox method). A wide variety of tumors was detected in positive controls.</p>	Moon et al. (1990)

5.0 GENOTOXICITY

Studies of the genotoxic effects of 1-NP are summarized in Table 5-1.

Summary: 1-NP was found to exhibit highly reproducible genotoxicity in a wide variety of prokaryotic and mammalian *in vitro* and *in vivo* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1989)]. When tested *in vitro*, 1-NP was found to induce DNA damage in *Escherichia coli*, *Salmonella typhimurium*, mouse hepatocytes, rat hepatoma cells, and Chinese hamster V79 and DON cells; gene mutations in *Escherichia coli*, *Salmonella typhimurium*, Chinese hamster ovary and V79 cells, mouse lymphoma cells, and human lymphocyte and hepatoma cells; unscheduled DNA synthesis (UDS) in mouse, rat, and hamster hepatocytes, and rat and human tracheal epithelial cells; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells; chromosomal aberrations and micronuclei in Chinese hamster lung fibroblasts; and cell transformation in mouse BALB/3T3 cells, Syrian hamster embryo cells, and human fibroblasts. *In vivo*, it was positive for DNA damage in mouse lungs, DNA adducts in rats, SCE in rat bone marrow, and mutagenic urine in rats. 1-NP was negative for gene conversions in *Saccharomyces cerevisiae*, DNA damage in rabbit lung Clara cells, DNA adducts in CHO K1 cells, and gene mutations in mouse lymphoma and V79 cells without S9.

Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*. Because of the extensive information available in IARC (1989) on the *in vitro* and *in vivo* genotoxicity of 1-NP, the post-1989 genotoxicity literature selection strategy was limited solely to studies that might offer additional unique information. In addition, for the sake of simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system were discussed as a group rather than cited individually.

5.1 Prokaryotic Systems

5.1.1 DNA Damage

As reported by IARC (1989), 1-NP was reported to induce DNA damage in *E. coli* [LED = 0.5 µg/mL (2 µM)] (Onta et al. 1984; cited by IARC, 1989) and *S. typhimurium* [LED = 0.02 µg/mL (0.08 µM)] (Nakamura et al. 1987; cited by IARC, 1989) in the absence of metabolic activation (see Genetic Activity Profile, Fig. 5-1). Sakai et al. (1985) reported that a 30-min preincubation with zinc acetate (240 µM) induced a 5-fold enhancement in the DNA-binding capacity of 1.5 µM 1-NP in *S. typhimurium* strain TA100 without metabolic activation. Oda et al. (1992) observed that *S. typhimurium* strain NM1011, containing plasmids with both a nitroreductase gene and the *umuC-lacZ* fusion gene, had a 3.8 fold higher β-galactosidase activity following 0.001 to 0.1 µg/mL (0.004 to 0.4 µM) 1-NP treatment than the untransformed parent strain. Lee et al. (1994) reported that 4000 nmol B[a]P/plate had an antagonistic effect on the production of DNA adducts induced by 100 nmol 1-NP/plate in strain TA98 mediated through altering the cell's nitroreductase metabolism.

5.1.2 Gene Mutations

1-NP [LED = 0.3 µg/plate (1.2 nmol/plate)] was also found to give positive results for gene mutations in *E. coli* without S9 activation (McCoy et al., 1985a, Tokiwa et al., 1984; cited by IARC, 1989). Stanton et al. (1988) found that 20 µM 1-NP induced a mutant frequency in *E. coli* strain AB1886 w/lambda phage c1857 about 2-fold higher than background. DNA

sequencing showed the mutations to be primarily single GC deletion frameshifts. Most recently, Malia et al. (1996) reported that *E. coli* strain GW5100, transformed with single-stranded M13 phage containing a dG-AP adducted CpG sequence, induced both competing -2 and +1 frameshift mutations in the M13 DNA.

In a study by Sakai et al. (1985), zinc acetate (240 μM) induced a 3-fold enhancement in the mutagenicity of 1.5 μM 1-NP in *S. typhimurium* strain TA100 without metabolic activation. Sato et al. (1985) reported that 1-NP was the predominant mutagen in the methanol extract of sediment from the Suimon River in Japan as detected in strains TA100, TA98, and TA98NR. Mumford et al. (1986) found that coal fly ash showed mutagenicity in strain TA98 only when coated by vapor phase deposition with 1-NP. As reported by IARC (1989) in 16 studies, 1-NP [LED = 0.01 to 0.30 $\mu\text{g}/\text{plate}$ (0.04 to 1.20 nmol/plate)] was found to give positive results for gene mutations in 8 strains of *S. typhimurium* (TA96, TA97, TA98, TA100, TA102, TA104, TA1537, and TA1538) both with and without S9 activation. Lee et al. (1994) reported that 12.5 to 400 nmol B[a]P/plate had an antagonistic effect on the mutagenicity induced by 5 nmol 1-NP/plate in strains TA98 and TA98NR, mediated through altering the cells' nitroreductase metabolism. Most recently, Scheepers et al. (1995) observed a relatively high correlation between the mutagenic potency of diesel exhaust particulates in acetone extracts of indoor air samples and their 1-NP content.

5.2 Lower Eukaryotic Systems

1-NP [HID = 500 $\mu\text{g}/\text{mL}$ (2000 μM)] did not induce an increase in gene conversions or recombinations in the yeast *Saccharomyces cerevisiae* (McCoy et al., 1983b, 1984; cited by IARC, 1989).

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Damage

As compiled by IARC (1989), 1-NP induced DNA damage as measured by alkaline elution in mouse hepatocytes [LED = 10 μM] (Moller and Thorgeirsson, 1985; cited by IARC, 1989). Chinese hamster DON [LED = 10 $\mu\text{g}/\text{mL}$ (40 μM)] (Edwards et al., 1986b; cited by IARC, 1989) and V79 cells [LED = 15 μM] (Saito et al., 1984b; cited by IARC, 1989) without S9, as well as in rat hepatoma cells [LED = 10 μM] (Moller and Thorgeirsson, 1985; cited by IARC, 1989). Becher et al. (1993), however, reported that 120 to 1200 μM 1-NP did not induce DNA damage (via alkaline elution) in rabbit lung Clara, type II, or macrophage cells.

IARC (1989) further reported that 1-NP was found to give positive results for induction of unscheduled DNA synthesis (UDS) in primary rat [LED = 1 μM] (Kornburst and Barfknecht, 1984; Mori et al., 1987; cited by IARC, 1989) mouse [LED = 14 μM] (Mori et al., 1987; cited by IARC, 1989) and hamster hepatocytes [LED = 10 μM] (Kornburst and Barfknecht, 1984; cited by IARC, 1989) and rat and human tracheal epithelial cells [LED = 10 μM] (Doolittle and Butterworth, 1984; Sugimora and Takayama, 1983; cited by IARC, 1989). It induced UDS in rabbit lung Clara, but not alveolar type II cells [LED = 1.25 $\mu\text{g}/\text{ml}$ (5.01 μM)] (Haugen et al., 1986 cited by IARC, 1989). In addition, Silvers et al. (1994) also reported that 4.0 μM 1-NP induced a 52% increase in UDS over controls in human hepatoma HepG2 cells exposed for 24 hours.

IARC (1989) also reported that 1-NP gave positive results for the induction of sister chromatid exchanges in Chinese hamster ovary cells both in the presence and absence of S9

activation [LED = 0.7 µg/mL (2.8 µM)] (Lewtas, 1982; Nachtman and Wolff, 1982; cited by IARC, 1989). Howard and Beland (1982) found that the mammalian nitroreductase, xanthine oxidase (0.05 to 0.5 U/mL), catalyzed the DNA binding of 20 µM [³H]1-NP to calf thymus DNA after a 4-hour exposure. Using the ³²P-postlabeling technique, Thornton-Manning et al. (1991a) reported that 40 µM 1-NP exposure for 24 hours induced DNA adducts [primarily *N*-(deoxyguanosin-8-yl)-1-aminopyrene (dG-AP)] in excision-repair-deficient UV5 CHO cells. In addition, Gallagher et al. (1993) also reported that both 20 µM 1-NP and diesel exhaust (100 µg soot/mL) induced DNA adducts in both human lymphocytes and calf thymus DNA. The major adduct formed from the diesel exhaust was chromatographically distinct from the 1-NP induced adduct.

Vyas and Basu (1995) placed a DNA 25mer oligonucleotide, adducted by treatment with 15 µM 1-NP, in a cell-free system with DNA polymerase to test DNA replication about the adducted site. The dG-AP adduct was found to efficiently block replication; however, when the DNA polymerase did manage to pass, the correct nucleotide (dCTP) was preferentially incorporated.

5.3.2 Gene Mutations

In a review by IARC (1989), 1-NP induced a positive mutagenic response (locus not provided) in Chinese hamster ovary cells [LED = 20 µg/mL (80 µM)] (Li and Dutcher, 1983; cited by IARC, 1989) and V79 cells [LED = 2 µM] (Berry et al., 1985; Ball et al., 1984; cited by IARC, 1989) and mouse lymphoma L5178Y cells [LED = 0.0 µM (doses were not given)] (Lewtas et al., 1982; cited by IARC, 1989) all only in the presence of metabolic activation, but did not induce an increase in revertants in Chinese hamster lung cells [HID = 20 µg/mL (81 µM)] (Nakayasu et al., 1982; cited by IARC, 1989). Thornton-Manning et al. (1991b) found that 2.5 to 15 µg/mL (10 to 60 µM) 1-NP did induce a significant mutagenic response in CHO K1-BH4 cells and in excision-repair-deficient UV5 cells. 1-NP was more mutagenic under a 5-hour anaerobic exposure than aerobic and greater in the UV5 cells, although the addition of flavin mononucleotide (FMN) to the S9 mix had no effect. In addition, Silvers et al. (1994) also reported that 10 µM 1-NP induced a 15-fold increase in *hprt* mutants over controls in human hepatoma HepG2 cells exposed for 24 hours.

5.3.3 Chromosomal Damage

As compiled by IARC (1989), 1-NP was found to give a positive result for chromosome aberrations in Chinese hamster lung fibroblasts in the absence of S9 [LED = 15 µg/mL (60 µM)] (Lafi and Parry, 1987; cited by IARC, 1989). Li et al. (1993) found 1-NP (62.5 to 1000 µM) to induce micronuclei in Chinese hamster lung cells exposed for 24 hours in the absence and 6 hours in the presence of S9 activation (LED = 62.5 µM +/-S9).

5.3.4 Morphological Transformation

As compiled by IARC (1989), 1-NP was found to give positive results for morphological transformation in Syrian hamster embryo cells [LED = 4.0 µM] (Dipaolo et al., 1983; cited by IARC, 1989) and human fibroblasts [LED = 3 µM] (Howard et al., 1983b; Kumari et al 1984; cited by IARC, 1989) in the absence of metabolic activation. Sheu et al. (1994) reported that 1-NP at 0.8 (LED) to 20 µg/mL (3 to 80 µM) for 48 hours induced morphological transformations in BALB/3T3 cells.

5.4 Mammalian Systems *In Vivo*

5.4.1 DNA Damage

As reviewed by IARC (1989), 1-NP induced DNA damage as measured by alkaline elution [LED = 50 mg/kg (200 μ mol/Kg) via intratracheal instillation] (Michell, 1984; cited by IARC, 1989) in mouse lungs. IARC (1989), also reported that 1-NP induced a slight increase in sister chromatid exchanges over controls in rat bone marrow [LED = 500 mg/kg (2000 μ mol/kg)] (Marshall et al., 1982; cited by IARC, 1989).

Beland et al. (1989) reported that 1-NP (doses not provided) induced DNA adducts as measured by 32 P postlabeling in the mammary gland, liver, and lungs of female Sprague Dawley rats. Bond et al. (1990) also reported that 1-NP induced DNA adducts in the lungs of male F344/N rats exposed via inhalation to 2 mg (8 μ mol) 1-NP/m³/4 hours/day 1 day/week for 12 weeks (a maximum peak was observed at 8 weeks).

5.4.2 Mutagenic Body Fluids

As reviewed by IARC (1989), male rats exposed to 1-NP produced mutagenic urine [LED = 10 mg/kg (40 μ mol/kg)] (Ball et al., 1984a; cited by IARC, 1989) in the presence of S9 and bile [LED = 4.0 mg/kg (16 μ mol/Kg)] (Morotomi et al., 1985; cited by IARC, 1989) in the presence and absence of S9 as tested in *S. typhimurium*.

5.5 Genotoxicity of 1-NP Metabolites

Studies of the genotoxic effects of 1-NP metabolites and derivatives are summarized in Table 5-2. Several other metabolite genotoxicity studies are mentioned in subsection 6.3.

5.5.1 1-Nitropyrene-4,5- and 8,9-oxide

Using the 32 P-postlabeling technique, Beland et al. (1992), reported that exposure to 1-NP and 2 of its metabolites, 1-nitropyrene-4,5- and -8,9-oxide (doses not provided) induced DNA adducts in both Chinese hamster ovary cells and calf thymus DNA, and *hprt* gene mutations in CHO cells. The same adducts [predominantly *N*-(deoxyguanosin-8-yl)-1-aminopyrene] were found in both CHO cells and calf thymus DNA treated with either metabolite.

5.5.2 4,5-Epoxy-4,5-dihdropyrene

Roy et al. (1991) tested 4,5-epoxy-4,5-dihdropyrene (20 to 40 μ M for 3 hours) for the induction of calf thymus DNA adducts using reverse-phase HPLC and found the metabolite to produce 3 major adduct peaks.

5.5.3 1-Nitrosopyrene

McGregor et al. (1994) reported that 0.75 to 1.0 μ M 1-nitrosopyrene increased the *hprt* mutant frequency in human T-lymphocytes 6 to 7 times over background. Single base substitutions were found in 63% of the mutants.

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Prokaryote Systems							
5.1.1 DNA Damage							
<i>Escherichia coli</i>	DNA damage	-	n.p.	n.g.	positive	LED = 0.5 µg/mL (2 µM)	Ohta et al. (1984; cited by IARC, 1989)
<i>Salmonella typhimurium</i>	DNA damage	-	n.p.	n.g.	positive	LED = 0.02 µg/mL (0.08 µM)	Nakamura et al. (1987; cited by IARC, 1989)
<i>S. typhimurium</i> strain TA100	DNA binding of [³ H]1-NP	-	n.p.	1.5 µM 1-NP plus 30 min. preincubation w/80-320 µM zinc acetate (ZA)	positive (+/- ZA)	Zinc ions (240 µM) induced a 5-fold enhancement in the DNA binding of 1.5 µM 1-NP.	Sakai et al. (1985)
<i>S. typhimurium</i> strains NM1011 containing plasmids with both a nitroreductase gene and the umuC-lacZ fusion gene	gene expression (β-galactosidase activity)	-	n.p.	0.001 to 0.1 µg/ml (0.004 to 0.4 µM)	positive	Strain NM1011 had a 3.8-fold higher β-galactosidase activity following 1-NP treatment than the parent (untransformed) strain.	Oda et al. (1992)
<i>S. typhimurium</i> strains TA98	DNA adducts (³² P positive labeling)	-	n.p.	100 nmol/plate +/- 250 to 4000 nmol/plate B[a]P	positive (+/- B[a]P)	B[a]P had an antagonistic effect on the DNA adducts induced by 1-NP mediated through altering its nitroreductive metabolism.	Lee et al. (1994)
5.1.2 Gene Mutations							
<i>E. coli</i>	gene mutations (locus not provided)	-	n.p.	n.g.	positive	LED = 0.3 g/plate (1 nmol/plate)	McCoy et al (1985a) and Tokiwa et al. (1984); both cited by IARC (1989)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
<i>E. coli</i> strain AB1886 with lambda phage c1857	lambda phage mutations	-	n.p.	20 µM for 90 min.	positive	1-NPO induced a mutant frequency ~2-fold higher than background. Sequencing showed the mutations to be primarily single GC deletion frameshifts.	Stanton et al. (1988)
<i>E. coli</i> strain GW5100 transformed with single stranded M13 phage containing a dG-AP adducted CpG sequence	types of mutagenesis (DNA sequencing)	-	n.p.	15 µmol for 16-20 h to adduct the CpG DNA. SOS induced in <i>E. coli</i> by 254 nm UV light.	positive	DG-AP induced both competing -2 and +1 frameshift mutations in the M13 DNA. The +2 frameshifts did not require SOS functions, but the +1 frameshifts were SOS dependent.	Malia et al. (1996)
<i>S. typhimurium</i> strain TA100	his gene mutations	-	n.p.	1.5 µM 1-NP plus 30 min. preincubation w/80-320 µM zinc acetate	positive (+/- ZA)	Zinc ions (240 µM) induced a 3-fold enhancement in the mutagenicity of 1.5M 1-NP.	Sakai et al. (1985)
<i>S. typhimurium</i> strains TA100, TA98, and TA98NR	his gene mutations	+/-	river sediment methanol extract	river sediment was 25.2 µg/kg 1-NP	positive/positive	1-NP was the predominant mutagen in the sediment from the Suimon River, Japan.	Sato et al. (1985)
<i>S. typhimurium</i> strain TA98	his gene mutations	-	1-NP and coal fly ash	vapor phase deposition of 1-NP onto coal fly ash particles (3 and 7 mg/plate)	positive	Coal fly ash showed mutagenicity only when coated with 1-NP.	Mumford et al. (1986)
<i>S. typhimurium</i> strains TA96, TA97, TA98, TA100, TA102, TA104, TA1537, and TA1538	his gene mutations	+/-	n.p.	n.g.	positive/positive	LED = 0.01 to 0.3 g/plate (0.04 to 1.20 nmol/plate), Salmonella results based on 8 strains over 14 papers	16 papers cited by IARC (1989)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
<i>S. typhimurium</i> strains TA98 and TA98NR	his gene mutations	-	1-NP and B[a]P, n.p.	1 to 8 nmol/plate +/- 40 nmol/plate B[a]P	positive (+/- B[a]P) in TA98 only	B[a]P had an antagonistic effect on the mutagenicity induced by 1-NP mediated through altering its nitroreductive metabolism.	Lee et al. (1994)
<i>S. typhimurium</i> strains TA98, TA1538, YG1021, and YG1024	his gene mutations	+/-	diesel exhaust particulate matter from various sources in indoor workplaces	1-NP content of acetone extracts determined via GC-MS analysis (0 to 0.8 ng/m ³)	positive (all strains)	A relatively high correlation was observed between mutagenic potency of the air sample extracts and the 1-NP content.	Scheepers et al. (1995)
5.2 Lower Eukaryote Systems							
<i>Saccharomyces cerevisiae</i>	gene conversions and recombinations	-	n.p.	n.g.	negative	HID = 500 g/mL (2000 µM)	McCoy et al. (1983b, 1984; cited by IARC (1989)
5.3 Mammalian Systems <i>In Vitro</i>							
5.3.1 DNA Damage							
mouse hepatocytes	DNA damage (alkaline elution)	-	n.p.	n.g.	positive	LED = 10 M	Moller and Thorgeisson (1985; cited by IARC, 1989)
Chinese hamster DON and V79 cells	DNA damage (alkaline elution)	-	n.p.	n.g.	positive	LED = 10 g/mL [40 M] (CH DON) and LED = 15 M (V79)	Edwards et al (1986b) and Saito et al (1984b); both cited by IARC (1989)
rat hepatoma cells	DNA damage (alkaline elution)	-	n.p.	n.g.	positive	LED = 10 M	Moller and Thorgeisson (1985; cited by IARC, 1989)
rabbit lung Clara, type II, and macrophage cells	DNA damage (alkaline elution)	-	n.p.	120 to 1200 µM	negative (all 3 cell types)	No significant increase for all three cell types.	Becher et al. (1993)
mouse hepatocytes	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 3.5 g/ml (14 M)	Mori et al. (1987; cited by IARC, 1989)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
rat hepatocytes	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 1 M	Kornburst and Barfknecht, (1984) and Mori et al. (1987); both cited by IARC (1989)
hamster hepatocytes	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 10 M)	Kornburst and Barfknecht (1984; cited by IARC, 1989)
rat tracheal epithelial cells	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 10 M	Doolittle and Butterworth (1984; cited by IARC, 1989)
human tracheal epithelial cells	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 10 M	Sugimura and Takayama (1983; cited by IARC, 1989)
Rabbit lung Clara cells	unscheduled DNA synthesis	-	n.p.	n.g.	positive	LED = 1.25 g/ml (5M)	Haugen et al. (1986; cited by IARC, 1989)
human hepatoma HepG2 cells	unscheduled DNA synthesis	-	n.p.	4 M for 24 hours	positive	1-NP induced a 52% increase over controls for UDS.	Silvers et al. (1994)
Chinese hamster ovary cells	sister chromatid exchanges	+/-	n.p.	n.g.	positive/positive	LED = 0.7 g/mL (2.8 M)	Lewtas (1982) and Nachtman and Wolff (1982); both cited by IARC (1989)
calf thymus DNA	DNA binding of [³ H]1-NP	-	n.p.	20 M [³ H]1-NP plus xanthine oxidase (0.05-0.5 U/mL) for 4 hours	positive	The mammalian nitroreductase, xanthine oxidase, catalyzed the binding of 1-NP to DNA.	Howard and Beland (1982)
Chinese hamster ovary excision repair deficient UV5 cells	DNA adducts (³² P postlabeling)	-	n.p.	40 M for 5-24 hours	positive (UV5)	Significant increase in adducts [N-(deoxyguanosin-8-yl)-1-aminopyrene] in UV5 cells exposed for 24 hours.	Thornton-Manning et al. (1991a)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
calf thymus DNA	DNA adducts (³² P postlabeling)	+	1-NP and diesel extracts, n.p.	20 M 1-NP or 100 g/mL soot plus 0.5 units/mL xanthine oxidase for 1.5 hours.	positive	The one major adduct formed by diesel exhaust was chromatographically distinct from the 1-NP induced adducts.	Gallagher et al. (1993)
human lymphocytes	DNA adducts (³² P positive labeling)	+	1-NP and diesel extracts, n.p.	20 M 1-NP or 100 g/mL soot for 1.5 hours.	positive	The one major adduct formed by diesel exhaust was chromatographically distinct from the 1-NP-induced adducts.	Gallagher et al. (1993)
5.3.2 Mutation Studies							
Chinese hamster ovary, V79, and lung cells	gene mutations (locus not provided)	+/-	n.p.	n.g.	positive/negative	Positive responses in CHO and V79 in the presence of S9 activation only. Negative response (+/-S9) only in CHL cells. LED = 20 g/mL [77 M] (CHO), 2 M (V79); HID = 20 g/mL [80 M] (CHL).	Li and Dutcher (1983), Ball et al. (1984), Berry et al (1985), and Nakayasu et al. (1982); all cited by IARC (1989)
Mouse lymphoma cells	tk gene mutations	+/-	n.p.	n.g.	positive/negative	None	Lewtas (1982; cited by IARC, 1989)
Chinese hamster ovary K1-BH4 and excision repair deficient UV5 cells	hprt gene mutations	+ (+/-FMN)	n.p.	2.5 to 15 g/mL (10 to 60 M) for 5 h aerobic and anaerobic	positive (both cell types)	1-NP was more mutagenic under anaerobic than aerobic conditions; greater in UV5 cells than K1-BH4 cells; and the addition of FMN in the S9 had no effect.	Thornton-Manning et al. (1991)
human hepatoma HepG2 cells	hgprt gene mutations	-	n.p.	10 M for 24 hours	positive	15 fold increase over controls for mutations.	Silvers et al. (1994)
Chinese hamster lung fibroblasts	chromosome aberrations	-	n.p.	n.g.	positive	LED = 15.0 g/mL (60 M)	Laffi and Parry (1987; cited by IARC, 1989)
Chinese hamster lung fibroblasts	micronuclei formation	+/-	n.p.	62.5 to 1000 M for 24 h (-S9) or 6 h (+S9)	positive/positive	LED = 62.5 M (+/-S9)	Li et al. (1993)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.3.4 Morphological Transformation							
BALB/3T3 cells clone A31-1-1	morphological transformation	-	n.p.	0.8 to 20.0 g/mL (3 to 80 M) for 48 hrs	positive	LED = 0.8 g/mL (3 M)	Sheu et al. (1994)
Syrian hamster embryo (SHE) cells	morphological transformation	-	n.p.	n.g.	positive	LED = 4 M	Dipaolo et al. (1983; cited by IARC, 1989)
human fibroblasts	morphological transformation	-	n.p.	n.g.	positive	LED = 3 M anaerobic	Howard et al. (1983b) and Kumari et al. (1984); both cited by IARC (1989)
5.4 Mammalian Systems <i>In Vivo</i>							
5.4.1 DNA Damage							
mouse (lungs)	DNA damage (alkaline elution)	NA	n.p.	n.g., exposure route not specified	positive	LED = 50 mg/kg (200 mol/Kg) via intratracheal instillation	Mitchell (1984; cited by IARC, 1989)
rats (bone marrow)	sister chromatid exchange	NA	n.p.	n.g., exposure route not specified	positive	Slight increase in SCEs, LED = 500 mg/kg (2000 mol/Kg)	Marshall et al. (1982; cited by IARC, 1989)
female Sprague Dawley rats	DNA adducts (³² P-postlabeling)	NA	99.9%	n.g., exposure route not specified	positive	Adducts were found in mammary gland DNA, liver DNA, and lung DNA.	Beland et al. (1989)
male F344/N rats	DNA adducts in lung (³² P-postlabeling)	NA	diesel exhaust and 1-NP, n.p.	0.35-10 mg soot/m ³ 7 hours/day, 5 days/week for 12 weeks or 2 mg (8mol) 1-NP/m ³ 4 hours/day, 1 day/week for 12 weeks	positive for both 1-NP and soot	1-NP DNA adducts reached a maximum 8 weeks into the exposure indicating lung tissue is metabolically capable of activating 1-NP.	Bond et al. (1990)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
male rats (species not provided)	mutagenic urine and bile (via Ames Test)	+/-	n.p.	n.g., exposure route not specified	positive/positive	Urine was mutagenic in the presence of S9 and bile was mutagenic both with and without S9. LED = 10.0 mg/kg [40 µmol/kg] (urine), 4.0 mg/kg [15 µmol/kg] (bile)	Ball et al. (1984a) and Morotomi et al. (1985); both cited by IARC, 1989
5.5 Genotoxicity of 1-NP Metabolites							
Calf thymus DNA	DNA adducts (³² P-postlabeling)	-	1-NP metabolites 1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide.	n.g.	positive (both metabolites)	The same adducts [predominantly <i>N</i> -(deoxyguanosin-8-yl)-1-aminopyrene] were found in calf thymus DNA treated with either metabolite.	Beland et al. (1992)
Chinese hamster ovary cells	DNA adducts (³² P-postlabelling)	-	1-NP metabolites 1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide.	n.g.	positive (both metabolites)	The same adducts [predominantly <i>N</i> -(deoxyguanosin-8-yl)-1-aminopyrene] were found in CHO cells treated with either metabolite.	Beland et al. (1992)
Chinese hamster ovary cells	<i>hprt</i> gene mutations	-	1-NP metabolites 1-nitropyrene-4,5-oxide and 1-nitropyrene-9,10-oxide.	10 nmol/mL.	positive (both metabolites)	A similar mutagenic response was seen in CHO cells treated with either metabolite	Beland et al. (1992)

Table 5-1. Summary of 1-Nitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
Calf thymus DNA	DNA adducts (reverse phase HPLC)	-	1-NP metabolite 4,5-epoxy-4,5-dihydro-1-nitropyrene, n.p.	no; 20 to 40 μ M for 3 hours	positive	Three major adduct peaks were obtained.	Roy et al. (1991)
human T-lymphocytes	<i>hprt</i> gene mutations	-	1-NOP, n.p.	0.75 and 1.0 μ M for 1 hour.	positive	1-NP increased the mutant frequency 6-7 times over background. Single base substitutions were found in 63% of the mutants.	McGregor et al. (1994)
DNA 25mer oligonucleotide containing a single dG-AP adduct	DNA replication through adduct site	-	1-NOP, n.p.	15 μ M	positive	The adduct blocked replication, but when the polymerase did pass the dG-AP adduct, the correct nucleotide (dCTO) was preferentially incorporated.	Vyas and Basu (1995)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided

Figure 5-1. Genetic Activity Profile of 1-Nitropyrene
(Data limited to IARC, 1989)

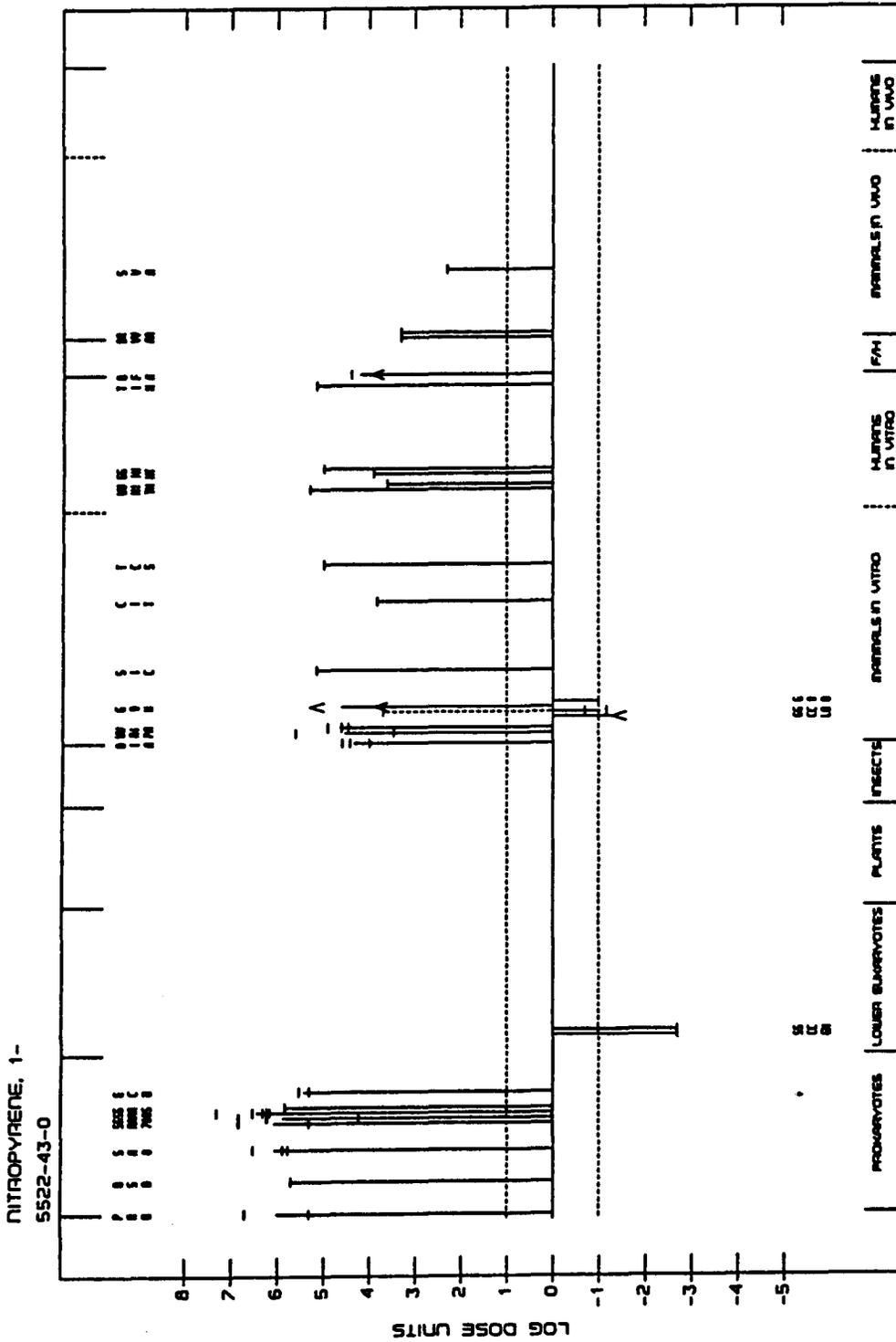
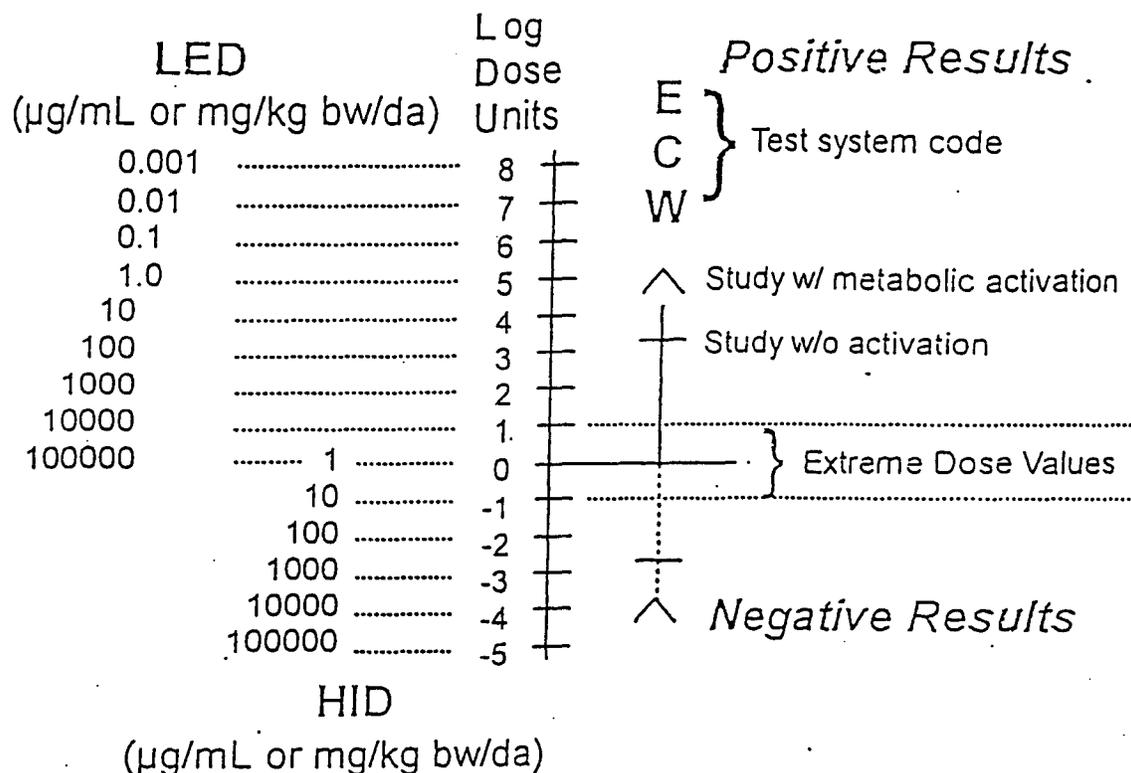


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. *Mutat. Res.* 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. *Mutat. Res.* 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. *Environ. Health Perspect.* 96:41-45.

6.0 OTHER RELEVANT STUDIES

6.1 Absorption, Distribution, Metabolism, and Excretion

Summary: A physiologically based toxicokinetic model was developed describing the disposition of 1-NP in rats after oral or inhalation exposure of 1-NP to rats. First order rate constants for absorption of 1-NP from the GI tract (2 h^{-1}) and covalent binding of 1-NP to tissue macromolecules (0.05, 0.05, 0.1, and 0.001 h^{-1} for lung, liver, kidney, and general tissue compartment, respectively) were determined from model simulations. The metabolic fate of 1- ^{14}C]NP was studied in rats dosed i.p. with 1- ^{14}C]NP. Within 24 h of dosing, 40% of the dose was found in feces and 15% in urine. Rats administered radiolabeled 1-NP i.p., orally, or by intragastric gavage excreted approximately 70 to 80% of the radiolabel in urine and feces within 96 h. Biliary excretion was the major route of excretion in bile duct-cannulated rats. In the feces, the major 1-NP metabolites were identified as 1-aminopyrene, 1-amino-6-hydroxypyrene, and 1-amino-8-hydroxypyrene. Hydroxy-*N*-acetyl-1-aminopyrene (6 and 8 isomers) was also identified in the feces. Urinary metabolites included 1-amino-3-, -6-, and -8-hydroxypyrene as well as 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene.

1-NP is metabolized by xanthine oxidase, DT-diaphorase, or aldehyde oxidase in mammalian systems under anaerobic conditions or by nitroreductases in bacterial systems. Nitroreduction of 1-NP leads to the formation, in sequence, to the corresponding 1-nitrosopyrene (1-NOP), *N*-hydroxy-1-aminopyrene, and 1-aminopyrene (1-AP). Acetylation by AcCoA of (IV) results in the formation of *N*-acetyl-1-aminopyrene. *N*-hydroxy-1-aminopyrene has been postulated by Beland (1991) to undergo spontaneous decomposition to reactive aryl nitrenium ion capable of covalently binding with DNA at the C-8 of guanine to give/form *N*-(deoxyguanosin-8-yl)-1-aminopyrene (C8-dG-AP) DNA adduct. In addition to the formation of this DNA adduct, two *N*²-deoxyguanosine adducts, 6-(deoxyguanosin-*N*²-yl)-1-AP and 8-(deoxyguanosin-*N*²-yl)-1-AP, were formed via nitroreduction.

Human liver microsomes derived from 16 individual liver specimens incubated with 1-NP yielded a range of metabolic rates from 0.13 to 0.99 nmol/min/mg protein, and the K_M for 3 microsomal samples with different V_{max} rates was 3.3-0.5 μM .

In the rat, after oral administration, 1-NP is either absorbed in the upper GI tract or is reduced to 1-AP by the intestinal microflora in the lower GI tract and is not believed to be absorbed. After absorption, 1-NP is oxidatively metabolized and conjugated in the liver. The conjugates are further metabolized by glucuronide cleavage and nitroreduction in the lower GI tract. The amine aglycones (unconjugated aminopyrenols) can then be reabsorbed, enter enterohepatic circulation, and be transported to the liver where they are *N*-acetylated, reconstituted, and excreted.

6.1.1 Absorption, Distribution, and Excretion

A physiologically based toxicokinetic model was developed describing the disposition of 1-NP in rats after oral or inhalation exposure of 1-NP to rats. First order rate constants for absorption of 1-NP from the GI tract (2 h^{-1}) and covalent binding of 1-NP to tissue macromolecules (0.05, 0.05, 0.1, and 0.001 h^{-1} for lung, liver, kidney, and general tissue compartment, respectively) were determined from model simulations (Medinsky et al., 1985). The metabolic fate of 1- ^{14}C]NP was studied in rats dosed i.p. with 1- ^{14}C]NP (10 mg/kg [40 $\mu\text{mol/kg}$]). Within 24 h of dosing, 40% of the dose was found in feces and 15% in urine. Rats

administered radiolabeled 1-NP i.p., orally, or by intragastric gavage excreted approximately 70 to 80% of the radiolabel in urine and feces within 96 h (Dutcher and Sun, 1983, cited by Chan, 1996; Ball et al., 1984; Ball and Lewtas, 1985; El-Bayoumy and Hecht, 1984). Medinsky et al. (1985) found that biliary excretion was the major route of excretion in bile duct-cannulated rats, with over 60% of the dose excreted in the bile within 24 h of dosing (i.v.). In the feces, the major 1-NP metabolites were identified as 1-aminopyrene, 1-amino-6-hydroxypyrene, and 1-amino-8-hydroxypyrene. Hydroxy-*N*-acetyl-1-aminopyrene (6 and 8 isomers) was also identified in the feces. Urinary metabolites included 1-amino-3-, -6-, and -8-hydroxypyrene as well as 6- and 8-hydroxy-*N*-acetyl-1-aminopyrene (Dutcher and Sun, 1983; Ball et al., 1984; El-Bayoumy and Hecht, 1984; Ball and Lewtas, 1985; Bond et al., 1986).

6.1.2 Metabolite and DNA Adduct Identification

Experimental details of the studies discussed in this section are presented in Table 6-1. Structures of many of the metabolites are shown in the metabolic pathways depicted in Figures 6-1 (Nitroreduction Pathway) and 6-2 (Ring Oxidation Pathway).

1-NP (I) is metabolized by xanthine oxidase, DT-diaphorase, or aldehyde oxidase in mammalian systems under anaerobic conditions or by nitroreductases in bacterial systems. Nitroreduction of 1-NP leads to the formation, in sequence, to the corresponding 1-nitrosopyrene (1-NOP) (II), *N*-hydroxy-1-aminopyrene (III), and 1-aminopyrene (1-AP) (IV). Acetylation by AcCoA of (IV) results in the formation of *N*-acetyl-1-aminopyrene (V). Compound III has been postulated by Beland (1991) to undergo spontaneous decomposition to reactive aryl nitrenium ion (VI) capable of covalently binding with DNA at the C-8 of guanine to give/form *N*-(deoxyguanosin-8-yl)-1-aminopyrene (C8-dG-AP) DNA adduct (XVI). In addition to the formation of XVI, two *N*²-deoxyguanosine adducts, 6-(deoxyguanosin-*N*²-yl)-1-AP and 8-(deoxyguanosin-*N*²-yl)-1-AP (XVII and XVIII, respectively), were formed via nitroreduction in incubations containing xanthine oxidase and 1-NP in the presence calf thymus DNA, and in DNA isolated from *S. typhimurium* suspension cultures incubated with 1-NP as well as in the mammary glands of female Sprague-Dawley rats administered 1-NP. The two *N*²-deoxyguanosinyl adducts were also formed in incubations containing rat liver cytosols and microsomes in the presence of calf thymus DNA.

Human liver microsomes derived from 16 individual liver specimens incubated with I yielded a range of metabolic rates from 0.13 to 0.99 nmol/min/mg protein, and the K_M for 3 microsomal samples with different V_{max} rates was 3.3-0.5 μ M. In these studies, P-450 3A was found to metabolize 60 to 70% of the microsomal metabolism of 1-NP. Human liver microsomal incubations differed from earlier rodent incubations in that human liver microsomes demonstrated the preference for the production of phenols at the C-3 position (VIII), whereas rodents preferentially formed 6-ol (IX) and 8-ol (X) over VIII. Urine samples collected from 3 mechanics exposed to 5.6 ng 1-NP/m³ (2.3×10^{-5} μ mol/m³) in a train engine repair shop revealed that the urinary metabolite 1-AP (IV) was significantly enhanced ($P < 0.05$) in mechanics when compared to office clerks. In the rat, after oral administration, 1-NP is either absorbed in the upper GI tract or is reduced to 1-AP (IV) by the intestinal microflora in the lower GI tract and is not believed to be absorbed. After absorption, 1-NP is oxidatively metabolized and conjugated in the liver. The conjugates are further metabolized by glucuronide cleavage (β -glucuronidase) and nitroreduction in the lower GI tract. The amine aglycones (unconjugated aminopyrenols)

can then be reabsorbed, enter enterohepatic circulation, and be transported to the liver where they are *N*-acetylated, reconstituted, and excreted (Kinouchi et al., 1986).

6.2 Pharmacokinetics

No data were available.

6.3 Modes of Action

Summary: In *S. typhimurium*, 1-NP is metabolically activated to a DNA binding species via nitroreduction and subsequent acetylation. However, in various mammalian systems, either ring oxidation or nitroreduction is capable of metabolically activating 1-NP. 1-NP is metabolically activated through cytochrome P-450-mediated C-oxidation. The mutagenicity of several epoxide, phenolic, and dihydrodiol derivatives of 1-NP in *S. typhimurium*, CHO cells, and rat hepatic microsomal preparations in the presence of calf thymus DNA correlated very well with the direct reaction of the epoxides with cellular DNA in these biological systems. In addition, nitroreduction of the phenolic or dihydrodiol metabolites to reactive *N*-hydroxaryl amines has also been reported to occur in these systems. Xanthine oxidase, DT-diaphorase, and aldehyde oxidase are capable of nitroreducing 1-NP to 1-NOP, 1-AP, or IV (reactive intermediates). Several studies have shown that mutations in mammalian and human *in vitro* systems and *S. typhimurium* correlate well with *N*-hydroxy-1-aminopyrene formation and subsequent DNA adduction. *In vitro* transformation of human diploid fibroblasts and Syrian hamster embryo cells has been similarly ascribed to this mode of action. It has been reported that incubation of CHO cells with 1-NOP produced a rapid concentration- and time-dependent induction of mutations. Thus, metabolic activation of 1-NP is responsible for genotoxicity in several mammalian systems and *S. typhimurium*.

Metabolic Activation and Genotoxicity

In *S. typhimurium*, 1-NP is metabolically activated to a DNA binding species via nitroreduction and subsequent acetylation (Messier et al., 1981; Quilliam et al., 1982; McCoy et al., 1983; Einisto et al., 1991; Kuo et al., 1992; Lee et al., 1994; for review see Table 6-1). However, in various mammalian systems, either ring oxidation or nitroreduction is capable of metabolically activating 1-NP (Nachtman and Wei, 1982; Saito et al., 1984; Beland et al., 1985; Howard et al., 1985; Djuric et al., 1986; Rozenkranz and Howard, 1986; Wolff et al., 1988; Belisario et al., 1989; Heflich et al., 1990; Kataoka et al., 1991; Howard et al., 1995; for review see Table 6-1).

1-NP is metabolically activated through cytochrome P-450-mediated C-oxidation (Guengrich et al., 1992; Gonzalez and Gelboin, 1994; for review see Table 6-1 and/or Howard et al., 1995) to XII and XIII with subsequent rearrangement to VII, VIII, IX, X, and XI (nitropyrenols) or hydration to XIV or XV (dihydrodiols) (Howard et al., 1995) (again numerals refer to those used in Figure 6-1). The mutagenicity of several epoxide, phenolic, and dihydrodiol derivatives of 1-NP in *S. typhimurium* (Consolo et al., 1989), CHO cells (Heflich et al., 1990), and rat hepatic microsomal preparations in the presence of calf thymus DNA (Djuric et al., 1986) correlated very well with the direct reaction of the epoxides with cellular DNA in these biological systems. In addition, nitroreduction of the phenolic or dihydrodiol metabolites to reactive *N*-hydroxaryl amines has also been reported to occur in these systems (Howard et al., 1995).

Xanthine oxidase (Bauer and Howard, 1990; Howard and Beland, 1982, cited by Howard et al., 1995), DT-diaphorase (Belisario et al., 1991; Silvers and Howard; cited by Howard et al., 1995), and aldehyde oxidase (Tatsumi et al., 1986, cited by Howard et al., 1995; Bauer and Howard, 1991) are capable of nitroreducing 1-NP to 1-NOP, 1-AP, or IV (reactive intermediates). Several studies have shown that mutations in mammalian and human *in vitro* systems and *S. typhimurium* correlate well with *N*-hydroxy-1-aminopyrene (III) formation and subsequent DNA adduction: *S. typhimurium* (Howard et al., 1983a), human hepatoma HepG2 cells (Eddy et al., 1987), and CHO cells (Heflich et al., 1986; Thornton-Manning et al., 1991a&b). In addition, *in vitro* transformation of human diploid fibroblasts (Howard et al., 1983b) and Syrian hamster embryo cells (DiPaolo et al., 1983) has been similarly ascribed to this mode of action (for review see Howard et al., 1995). It has also been reported that incubation of CHO cells with 1-NOP (II) produced a rapid concentration and time-dependent induction of mutations at the *hprt* locus (Heflich et al., 1986). Thus, metabolic activation of 1-NP is responsible for genotoxicity in several mammalian systems and *S. typhimurium*.

An extensive online search for NP-induced tumor studies was conducted using TOXLINE, CANCERLIT, EMBASE, and MEDLINE. However, no hits were found using the CASRN 5522-43-0 (1-NP) coupled with the keywords oncogene(s), RAS, MYC, NEU, or FAS.

6.4 Structure-Activity Relationships

The mutagenic and carcinogenic potentials of nitroarene analogues vary. Some analogues are mutagenic and genotoxic in many systems, while other analogues are only mutagenic in some systems or are not mutagenic at all (for review see Klopman and Rosenkranz, 1984). Furthermore, some analogues are carcinogenic in rodents, while other analogues are not (Rosenkranz, 1987).

Several studies have been performed that identify structure activity relationships among the nitroarenes. For example, Mermelstein et al. (1982) reported that there was an increase in the mutagenicity of nitropyrenes (exogenous metabolic activation not mentioned) towards *S. typhimurium* strains TA98 and TA98NR as the number of nitro groups per compound increased. Mutagenicity towards these 2 strains increased in the order: 1-NP < 1,3-DNP < 1,6-DNP < 1,8-DNP. However, with further addition of nitro groups, the mutagenic activity of nitropyrenes decreased. After reaching a maximum with 1,8-DNP, mutagenicity declined for 1,3,6-trinitro- and 1,3,6,8-tetranitropyrene. In other strains of *S. typhimurium* (TA1537 and TA1538), the mutagenicity of 1-NP was 2 to 3 orders of magnitude lower than the mutagenicities of di-, tri-, and tetranitropyrenes, but within the di-, tri-, and tetranitropyrenes there was no apparent pattern for increasing mutagenicity.

Klopman et al. (1984) reported that a linear relationship existed between the first half-wave potential ($E_{1/2}$) and the logarithms of the mutagenicities of various nitroarenes (including 1-NP, 1,6-DNP, and 1,8-DNP) towards *S. typhimurium* strains TA98 and TA1538. The lower the $E_{1/2}$, the more readily the nitroarene was nitroreduced. It was not stated whether exogenous metabolic activation was used. Since a linear relationship was also found to exist between $E_{1/2}$ and the calculated energies of the lowest unoccupied molecular orbital (LUMO), the authors suggested that the mutagenicities of nitroarenes could be predicted from their calculated LUMO energies. Debnath et al. (1992) reported that the mutagenic activity of aromatic and heteroaromatic nitro compounds (including 1-NP, 4-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA100, without exogenous metabolic activation, was also linearly related

to the LUMO energies of the compounds. Debnath et al. (1992) also reported that the mutagenicities of various nitroarenes were bilinearly related to the hydrophobicity of the compounds, with an optimal hydrophobicity constant (log P) of 5.44.

Klopman and Rosenkranz (1984) used the Computer Automated Structure Evaluation (CASE) program to predict the mutagenicity (without exogenous metabolic activation) of 53 nitroarenes (including 1-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA98. Two activating and 2 deactivating structures were reported to be involved in the mediation of nitroarene mutagenicity (see Figure 6-4).

6.5 Cell Proliferation

Full details of the cellular proliferation induced by 1-NP are provided in Table 6-2.

In 6-wk-old male F344 rats, oral administration once per day for 6 days of 100, 250, 500, or 1000 mg 1-NP per kilogram mean body weight (400B4000 $\mu\text{mol/kg}$) significantly increased the number of γ -glutamyltranspeptidase-positive foci in liver (Denda et al., 1989).

In 8-wk-old male F344/DuCrj rats, administration of 2 mg (8 μmol) 1-NP in 0.2 mL DMSO subcutaneously twice per week for 10 weeks induced the formation of inflammatory nodules and ulceration at the site of injection (Ohgaki et al., 1982). Both the nodules and ulcerations disappeared by day 100, and on day 162, the first injection-site tumor was detected. By the end of the study (when all rats were either moribund or dead), 1-NP-treated rats had a significantly higher incidence of injection-site tumors than did solvent controls.

In 30-day-old female CD rats, administration of 67 μmol 1-NP per kilogram mean body weight i.p. 3 times per week for 4 weeks did not induce the formation of hyperplastic nodules or altered foci in the liver (Imaida et al., 1991a).

In 7-week-old male and female F344/N rats, nose-only exposure 6 hours per day, 5 days per week, for 13 weeks to aerosol containing 2.0B200 μmol 1-NP failed to induce gross lesions; but the higher doses did induce microscopic lesions (squamous metaplasia) in the larynx (males and females) and lungs (males only) (Chan, 1996). Cytoplasmic alterations were also detected in the nasal tissues of males and females.

In 7- to 9-wk-old male Syrian golden hamsters, intratracheal instillation once or twice per week for 92 weeks of 1 or 2 mg 1-NP (4 or 8 μmol) adsorbed onto an equal mass of carbon carrier particles (Stokes diameter 2-5 μm , greater than 70%) increased the incidence of tracheal, pulmonary, and bronchiolar hyperplasia as compared to untreated controls (Moon et al., 1990).

6.6 Initiation/Promotion

In 50 to 55-day-old female CD-1 Charles River mice that received ten applications, on shaved dorsal skin, of 0.1 mg (0.4 μmol) 1-NP in 0.1 mL acetone, followed by 2.5 μg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL acetone 3 times per week for 25 weeks, there was no significant increase in the incidence of skin tumors (El-Bayoumy et al., 1982; cited by IARC, 1989).

In 7-week-old male and female SENCAR mice that received a single or double (3.0-mg group) dermal application of 0-3.0 mg (0-12 μmol ; individual doses not given) 1-NP in 0.2 mL acetone, followed 1 week later with skin applications of TPA (dose not given) in 0.2 mL acetone twice per week for 30 weeks, there was no significant increase in the incidence of skin tumors (Nesnow et al., 1984; cited by IARC, 1989).

Table 6-1. 1-Nitropyrene and Adduct Identification

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Nitrosopyrene [86674-51-3] (II)		CHO cells inefficient (Heflich et al., 1984). Rat liver cytosol (Howard et al., 1985).		Xanthine oxidase (Heflich et al., 1986) ^b .	In the presence of xanthine oxidase, calf thymus DNA produced the DNA adduct XVI (Heflich et al., 1986).
N-Hydroxy-1- aminopyrene; 1- Hydroxylaminopyrene (III)	Rat feces (El-Bayoumy and Hecht, 1984).		<i>S. typhimurium</i> (Rozenkranz and Mermelstein, 1985; Tokiwa and Ohnishi, 1986)	Undergoes spontaneous decomposition to form the corresponding nitrenium ion, which reacts with DNA to give the adduct XVI as postulated by Beland (1991).	

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Aminopyrene; 1-AP [1606-67-3] (IV)	<p>Human urine (Scheepers et al., 1994).</p> <p>Rat (conventional) feces and urine via i.p. injection, i.g., or orally (El-Bayoumy et al., 1983; Ball et al., 1984; El-Bayoumy and Hecht, 1984; Kinouchi et al., 1986; Ball and Lewtas, 1985; Dutcher and Sun, 1983, cited by Chan, 1996).</p> <p>Mice (homogenized whole; 1-day old) (El-Bayoumy et al., 1988).</p>	<p>Human erythrocytes and liver microsomes (Belisario et al., 1996)</p> <p>Mouse lung and liver explants and S9 (El-Bayoumy and Hecht, 1984).</p> <p>Rat liver fractions: S9, cytosol, and microsomes (Nachtman and Wei, 1982; Saito et al., 1984; Beland et al., 1985; Howard et al., 1985; Djuric et al., 1986; Belisario et al., 1989).</p> <p>Chinese hamster (CH) V79 lung fibroblasts and rat liver supernatants (El-Bayoumy and Hecht, 1983; Bond et al., 1984; Ball et al., 1985).</p> <p>Rabbit and hamster liver and lung microsomes and lung S9 (Dybing et al., 1986; King et al., 1984).</p>	<i>S. typhimurium</i> TA98 (Messier et al., 1981).	<p>Human hemolysate (Belisario et al., 1996).^b</p> <p>Cytosolic nitroreductase activity due to DT-diaphorase, aldehyde oxidase, xanthine oxidase, and other unidentified nitroreductases (Nachtman and Wei, 1982; Saito et al., 1984; Beland et al., 1985; Howard et al., 1985).^b</p> <p>Microsomal nitroreductase due to cytochrome P-450 reductase and cytochrome P-450.</p> <p>Oxygen inhibited reductive metabolism with an increase in 2 phenolic peaks (Saito et al., 1984; Beland et al., 1985; Djuric et al., 1986; Belisario et al., 1989).^b</p>	<p>Neither IV nor its conjugates found in urine of either conventional or germ-free rats (El-Bayoumy et al., 1983).</p> <p>Minimal formation of IV (El-Bayoumy and Hecht, 1984).</p> <p>Maximum metabolism observed in presence of FMN and NADPH (Nachtman and Wei, 1982; Saito et al., 1984; Beland et al., 1985).</p> <p>IV only detected metabolite (Saito et al., 1984). Induction of cytochromes P-450 with 3-methylcholanthrene increased rates of IV formation (Saito et al., 1984; Beland et al., 1985; Djuric et al., 1986; Belisario et al., 1989).</p> <p>The principal metabolite detected in anaerobic incubations including 1-NP and CHO cells. Under oxidative conditions, amine formation suppressed. (Heflich and Beland, unpub., cited by Beland et al., 1985). Not detected in 8- or 15-day old mice (El-Bayoumy and Hecht, 1988).</p>

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Aminopyrene; 1-AP [1606-67-3] (IV) (contd.)	Rat urine (Belisario et al., 1987).	Human, rat and mouse intestinal microflora (King et al., 1990). Rabbit, rat, and hamster tracheal epithelial cells (King et al., 1993).			<i>N</i> -Formyl-1-AP detected at very low concentrations (1 to 1.4% of dose) (King et al., 1990).
<i>N</i> -Acetyl-1-aminopyrene; 1-Acetamidopyrene; 1-Acetylaminopyrene [22755-15-3] (V)	Rat feces (El-Bayoumy and Hecht, 1984). Rat urine (Belisario et al., 1987).	Human, rat, and mouse intestinal microflora (King et al., 1990). Perfused rat liver preparations (Bond and Mauderly, 1984), CH V79 lung fibroblasts, and rat liver supernatants (El-Bayoumy and Hecht, 1983; Bond et al., 1984; Ball et al., 1985). Rat liver microsomes (Belisario et al., 1989). Rabbit lung S9 (King et al., 1984). Rabbit, rat, and hamster tracheal cells (King et al., 1993).	<i>S. typhimurium</i> TA98 (Messier et al., 1981).	Substrate for microsome-mediated (cytochrome P-450) ring hydroxylation (Belisario et al., 1989) ^c .	Similar mechanism involved in biotransformation of 1-NP by intestinal microflora in human, rat, and mouse (Cerniglia, 1984; Kinouchi et al., 1982; Kinouchi and Ohmishi, 1983, 1986; Manning et al., 1986; King et al., 1990).

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
Pyrenylnitrenium Ion (VI)			<i>S. typhimurium</i>	Acid catalyzed.	Aryl nitrenium ion covalently bonded with DNA to form XVI adduct (Howard et al., 1983). Capable of covalently bonding to DNA at the C-8 of guanosine, forming the adduct XVI (McCoy et al., 1983; Heflich et al., 1985; Howard and Beland, 1982).
Ring Oxidation					
1-Nitropyrene (I)	Mice, amniotic fluid, fetuses, and suckling neonates (Howard et al., 1995).	Rabbit liver microsomes (Howard et al., 1994).		Human P-450 3A (K_M , 3.3 0.5 μ M; V_{max} 0.13 to 0.99) nmol/min/mg; Silvers et al., 1992) ^c . Cytochrome P-450 2C3 (Park et al., 1988; Howard et al., 1994) ^c .	Human liver microsomes showed a preference for the formation of phenols at the C3 position, contrasting the preference of rodents, which preferentially formed 6-ol and 8-ol over the 3-ol metabolites (see; Rozenkranz and Howard, 1985; Djuric et al., 1986; Howard et al., 1987; Silvers et al., 1992). These findings are in agreement with Howard et al. (1990). Cytochrome P-450 2C3 is responsible for ~75% of the CYP450-mediated C-oxidation of 1-NP in rabbit liver (Howard et al., 1994). Accumulation of 1-NP and/or its metabolites in fetuses, amniotic fluid and suckling neonates occurred after treating pregnant mice by gavage with 1-NP (Howard et al., 1995).

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Hydroxypyrene; Pyrenol; Pyren-1-ol; [78751-40-3] (VII)		Rat liver microsomes (Djuric et al., 1986).			1-Pyrenol and 10-OH-1-NP have nearly identical retention times (HPLC).
3-Hydroxy-1-nitropyrene; 1-Nitropyren-3-ol; [86674-49-9] (VIII)	Rat urine and feces (Ball et al., 1984; Ball and Lewtas, 1985; Bond et al., 1986). Rat (germ-free) feces and urine (El-Bayoumy and Hecht, 1984b; Kinouchi et al., 1986). Mice (ground) (El- Bayoumy et al., 1988).	Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990). Rat liver extracts (El- Bayoumy and Hecht, 1983 and 1984b). Rat isolated perfused and ventilated lungs (Bond et al., 1984). Rat liver, lung, and nasal tissues and nasal S9 (Bond et al., 1983). Rabbit, rat, and hamster liver and lung microsomes and S9 (Dybing et al., 1986; Djuric et al., 1986; Howard et al., 1985; King et al., 1984).	<i>S. typhimurium</i> (Bond et al., 1983).	Cytochrome P-450 IIIA3 and IIIA4 preferentially metabolized 1-NP to the 3-, 6-, and 8- phenol metabolites (Howard et al., 1990) ^b .	In humans, 1-nitropyren-3-ol formed as the principal metabolite, produced in at least 4-fold greater abundance than the mixture of 1-nitropyren-6-, and 8-ol, or the K-region (4,5- and 9,10-) dihydrodiols. This is in contrast to rabbit and rat in which metabolism is catalyzed primarily by the P- 450I and P-450IIB and IIC enzymes (Howard et al., 1990). El-Bayoumy and Hecht, (1984b) conflict with El- Bayoumy and Hecht, (1984a), the former suggesting the involvement of intestinal microflora in the production of amino-hydroxypyrenes.

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
6-Hydroxy-1-nitropyrene; 1-Nitropyren-6-ol [1767-28-8] (IX)	<p>Rat (germ-free) feces and urine (El-Bayoumy and Hecht, 1984b; Kinouchi et al., 1986).</p> <p>Mice (ground) (El-Bayoumy et al., 1988).</p>	<p>Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990).</p> <p>Rat liver extracts (El-Bayoumy and Hecht, 1983 and 1984).</p> <p>Rat isolated perfused and ventilated lungs (Bond et al., 1984).</p> <p>Rat liver and nasal S9 (Bond, 1983; Belisario et al., 1986).</p> <p>Rat liver, lung, and nasal tissues (Bond et al., 1983).</p> <p>Rabbit, rat, and hamster liver and lung microsomes and S9 (Dybing et al., 1986; Djuric et al., 1986; Howard et al., 1985; King et al., 1984).</p>	<p><i>S. typhimurium</i> (Bond et al., 1983).</p> <p><i>Cunninghamella elegans</i> (Cerniglia et al., 1985)</p>	<p>Cytochrome P-450 IIIA3 and IIIA4 preferentially metabolized 1-NP to the 3-, 6-, and 8- hydroxy metabolites (Howard et al., 1990)^b.</p>	

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
8-Hydroxy-1-nitropyrene [1732-29-2] (X)	<p>Rat (germ-free) feces and urine (El-Bayoumy and Hecht, 1984b; Kinouchi et al., 1986).</p> <p>Rat urine and feces (Ball et al., 1984; Ball and Lewtas, 1985; Bond et al., 1986).</p> <p>Mice (homogenized whole) (El-Bayoumy et al., 1988).</p>	<p>Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990).</p> <p>Rat liver extracts (El-Bayoumy and Hecht, 1983 and 1984).</p> <p>Rat isolated perfused and ventilated lungs (Bond et al., 1984).</p> <p>Rat liver and nasal S9 (Bond, 1983; Belisario et al., 1986).</p> <p>Rat liver, lung, and nasal tissues (Bond et al., 1983).</p> <p>Rabbit, rat, and hamster liver and lung microsomes and S9 (Dybing et al., 1986; Djuric et al., 1986; Howard et al., 1985; King et al., 1984).</p>	<p><i>S. typhimurium</i> (Bond et al., 1983).</p> <p><i>Cunninghamella elegans</i> (Cerniglia et al., 1985)</p>	<p>Cytochrome P-450 IIIA3 and IIIA4 preferentially metabolized 1-NP to the 3-, 6-, and 8- hydroxy metabolites (Howard et al., 1990)^b.</p>	

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
10-Hydroxy-1-nitropyrene; 1-Nitropyren-10-ol; 10-OH- 1-NP [91254-92-1] (XI)		Rabbit liver S9 (King et al., 1984). Rat perfused lung (Bond and Mauderly (1984). Rabbit, rat, and hamster tracheal epithelial cells (King et al., 1987; King et al., 1993).			
1-Nitropyrene-4,5-oxide; 1- NP-4,5-epoxide [102822-04-8] (XII)	Human > dog > guinea pig > hamster > rat > mouse in hydration of 1- NP-4,5-oxide (Kataoka et al., 1991). Guinea pig (Fifer et al., 1986).	Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990). Rat and hamster liver microsomes (Dybing et al., 1986; Djuric et al., 1986).		Cytochrome P-450 IIIA3 and IIIA4 (Howard et al., 1990b).	Due to the identification of K- region epoxides and dihydrodiols (Howard et al., 1990). "...the principal route of detoxification of animals is P-450-mediated C-oxidation" (Howard et al., 1990). Oxides were not found in rabbit, rat, and hamster lung microsomes incubated with I or rabbit liver incubations. No single species studied was a good model for humans, and the balance of activation/inactivation was found to tend toward detoxification in adult animals studied (Kataoka et al., 1991).

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Nitropyrene-9,10-oxide; 1-NP-9,10-epoxide [105596-43-8] (XIII)	Guinea pig (Fifer et al., 1986).	Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990). Rat and hamster liver microsomes (Dybing et al., 1986; Djuric et al., 1986).		Cytochrome P-450 IIIA3 and IIIA4 (Howard et al., 1990b).	Due to the identification of K-region epoxides and 1-NP-4,5-dihydrodiol, epoxide hydrolase was implicated as the enzyme responsible for catalyzing this reaction. A...the principal route of detoxification of animals is P-450-mediated C-oxidation (Howard et al., 1990).
1-Nitropyrene-trans-9,10-dihydrodiol (XIV)		Rabbit liver and lung S9 (King et al., 1984). Rabbit, rat, and hamster liver and lung microsomes (Dybing et al., 1986; Djuric et al., 1986). Rabbit, rat, and hamster tracheal epithelial cells (King et al., 1993).		Epoxide hydrolase (Djuric et al., 1986b).	

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Nitropyrene-trans-4,5-dihydrodiol [86674-50-2] (XV)	<p>Rat (germ-free) feces and urine (El-Bayoumy et al., 1984; Kinouchi et al., 1986).</p> <p>Mice (ground) (El-Bayoumy et al., 1988).</p>	<p>Vaccinia virus expression of P-450 cDNAs in human HepG2 cells incubated with 1-NP (Howard et al., 1990).</p> <p>Rat liver extracts (El-Bayoumy and Hecht, 1983 and 1984; King et al., 1984; Dybing et al., 1986).</p> <p>Rabbit, rat, and hamster liver and lung microsomes (Dybing et al., 1986; Djuric et al., 1986).</p> <p>Mouse lung and liver explants and S9 (El-Bayoumy and Hecht, 1984).</p> <p>Rabbit lung S9 (King et al., 1984).</p>		Epoxide hydrolase (Howard et al., 1990 and Djuric et al., 1986b).	Discussed above for XIV.

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
1-Amino-3-hydroxypyrene [103114-40-5]	Rat urine (Ball et al., 1984)				
1-Amino-6-hydroxypyrene [1732-30-5]	Rat feces and urine (Ball et al., 1984, El-Bayoumy and Hecht, 1984a)				Rat intestinal microflora involved in the formation of 1-amino-6, and 8-hydroxypyrene (El-Bayoumy et al., 1984a).
1-Amino-8-hydroxypyrene [1732-31-6]	Rat feces and urine (El-Bayoumy and Hecht, 1984a).				Rat intestinal microflora involved in the formation of 1-amino-6, and 8-hydroxypyrene (El-Bayoumy et al., 1984a).
3-Hydroxy-N-acetyl-1-aminopyrene	Rat feces (Kinouchi et al., 1986)				
6-Hydroxy-N-acetyl-1-aminopyrene	Rat urine and feces (Ball et al., 1984; El-Bayoumy and Hecht, 1984a; Ball and Lewtas, 1985; Bond et al., 1986; Kinouchi et al., 1986; Ball et al., 1991).				Both nitroreduction and hydrolysis of glucuronides released for enterohepatic recirculation are important in generating mutagenic metabolites of 1-NP (Ohnishi et al., 1990; Ball et al., 1991).

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
8-Hydroxy-N-acetyl-1-aminopyrene	Rat urine and feces (Ball et al., 1984; El-Bayoumy and Hecht, 1984a; Ball and Lewtas, 1985; Bond et al., 1986; Kinouchi et al., 1986).				<p>Nitrohydroxypyrenes formed in the liver were conjugated to glucuronic acid and excreted by the bile duct into the intestines, and contribution of the intestines to acetylation of the reduced metabolites was very low (Kinouchi et al., 1987).</p> <p>Pancreatic juice plays an important role in the metabolism of glutathione conjugates to corresponding cysteine conjugates in the small intestine (Kinouchi et al., 1987) and the intestinal microflora are involved in the activation of metabolites in the intestine (Kinouchi et al., 1993).</p>
1-Nitro-5H-phenanthro[4,5-bcd]pyran-5-one		Rat liver S9 (Roy et al., 1988).			Lactones were not identified in these studies, and in subsequent incubations including the lactone and rat liver S9, HPLC analysis of the ethanol-acetate-extractable metabolites indicated that the substrate was recovered unchanged (Roy et al., 1988).

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
<i>N</i> -(Deoxyguanosin-8-yl)-1-aminopyrene; C8-dG-1-AP [85989-43-1] (XVI)	<p>Rat kidney, liver, lung, mammary gland, and peripheral blood lymphocytes (Hashimoto and Shudo, 1985; Stanton et al., 1985, cited by Chan, 1996; El-Bayoumy et al., 1988; Bond et al., 1990; Smith et al., 1990).</p> <p>Mouse lung and liver (Mitchell, 1988; El-Bayoumy et al., 1988; Smith et al., 1990).</p> <p>Rat (female) mammary fat pads and livers (Roy et al., 1989).</p>	<p>In the presence of rat liver S9, CHO cells (Heflich et al., 1986), Chinese hamster V79 cells (Patton et al., 1986), and Chinese hamster lung cells (CHL) (Nakayasu et al., 1982; Sugimura and Takayama, 1983). Human HepG2 cells (Howard, unpublished, cited by Howard, 1990), and human foreskin fibroblasts (Beland et al., 1986).</p> <p>Chinese hamster lung cells and calf thymus DNA incubated with xanthine oxidase and I or II (Edwards et al., 1986; Herreno-Saenz et al., 1995).</p> <p>Rabbit tracheal explants (Jeffrey et al., 1990)</p> <p>Single-stranded M13mp18 DNA treated with tritiated 1-nitrosopyrene (Malia and Basu, 1994).</p> <p>Calf thymus DNA and III (Herreno-Saenz, 1994).</p>	<p><i>S. typhimurium</i> (Howard et al. 1983).</p> <p>Bacteriophage M13mp18 DNA in the presence of II and ascorbic acid (Malia and Basu, 1994).</p>	<p>Xanthine oxidase (Edwards et al., 1986; Roy et al., 1989).</p>	<p>In vitro nitroreduction of 1-NP following sequential nitroreduction leads to formation of 1-nitrosopyrene (II), <i>N</i>-hydroxy-1-aminopyrene (III), 1-aminopyrene (IV), and the hydroxylamino (II) derivative forms XVI in the referenced biological systems (Howard et al., 1990). However, the major DNA adducts formed in vivo did not appear to form from simple nitroreduction, suggesting that other metabolic pathways may be involved in 1-NP DNA adducts formed in vivo (Roy et al., 1989).</p> <p>1-AP (IV) was inactive in incubations including xanthine oxidase and calf thymus DNA. However, in CHL cells, IV induced the formation of an unidentified DNA adduct (Edwards et al., 1986).</p> <p>Apigenin and tannic acid inhibited the nitroreductase activity in <i>S. typhimurium</i>, leading to inhibited bacterial mutagenesis when incubated with 1-NP (Kuo et al., 1992).</p>

Table 6-1. 1-Nitropyrene and Adduct Identification (Continued)

Metabolite Name [CASRN] Number ^a	In Vivo	In Vitro	Bacteria, Fungus	Enzymes	Comments
6-(Deoxyguanosin-N ² -yl)-1-Aminopyrene (XVII)	Rat (female) mammary gland (Herreno-Saenz et al., 1995).	Calf thymus DNA and III (Herreno-Saenz et al., 1995). Rat liver cytosol or microsomes in the presence of calf thymus and <i>S. typhimurium</i> DNA incubated with III or 1-NP (Herreno-Saenz et al., 1995).		Xanthine oxidase (Herreno-Saenz et al., 1995).	
8-(Deoxyguanosin-N ² -yl)-1-Aminopyrene (XVIII)	Rat (female) mammary gland (Herreno-Saenz et al., 1995).	Rat liver cytosol or microsomes in the presence of calf thymus and <i>S. typhimurium</i> DNA in incubated with III or 1-NP (Herreno-Saenz et al., 1995). Calf thymus DNA and III (Herreno-Saenz et al., 1995).		Xanthine oxidase (Herreno-Saenz et al., 1995).	

Table 6-2. Cell Proliferation Induced by 1-Nitropyrene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Oral Administration							
6-wk-old F344 rats	M (number not given)	M (corn oil alone; number not given)	1-NP, no di- and tri-nitropyrenes were detected by HPLC	100, 250, 500, or 1000 mg/kg bw (400B4000 μ mol/kg), once/day All rats (treated and control) underwent partial (2/3) hepatectomy (PH) 4 h after the fourth treatment. Another group was given one dose of 1000 mg/kg (4000 μ mol/kg) and underwent PH 4 h later.	6 days	All animals were killed 5 weeks after the last treatment. Liver: Positive (for γ -glutamyltranspeptidase-positive foci) The number of γ -glutamyltranspeptidase-positive foci (number/cm ² and number/cm ³) in liver was significantly increased in rats given multiple doses of 1-NP, but not in those that received only a single dose of 1-NP (Student's t-test).	Denda et al. (1989)
Rats - Subcutaneous Injection							
8-wk-old F344/DuCrj rats	20M	20M (DMSO alone)	1-NP, > 99% pure	2 mg (8 μ mol) in 0.2 mL DMSO, twice/wk	10 wk	All rats were observed until moribund or dead. Injection Site: Positive (for inflammatory nodules, ulceration, and tumorigenesis) All 1-NP-treated rats, but none of the vehicle controls, developed inflammatory nodules at the injection site during treatment. Thirty percent of these rats also displayed injection-site ulceration during treatment. Both the nodules and ulcerations disappeared by about day 100. On day 162, the first injection-site tumor was detected. At the end of the study, treated rats had a significantly increased incidence of injection-site tumors ($p < 0.003$; χ^2 test).	Ohgaki et al. (1982)

Table 6-2. Cell Proliferation Induced by 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Intraperitoneal Injection							
7-wk-old F344/N rats	10M, 10F per dose	10M, 10F (air only)	1-NP, >99% pure	0.5, 2, 8, 20, or 50 mg/m ³ (2.0B200 μmol/m ³), administered nose-only, 6 h/day, 5 days/wk	13 wk	<p>Rats were killed when they were 20 weeks old. All rats survived the 13-week treatment period. All major organs and tissues of the 50-mg dose group were examined histologically. In the lower dose groups, the larynx, lungs, and nasal cavity were examined histologically. None of the rats developed gross lesions, but microscopic lesions in larynx, lung, and nose were detected. Statistical analysis of incidence was performed using the Fisher exact test.</p> <p>Larynx: Positive (for metaplasia) Males that received doses of 2 mg/m³ (8.1 μmol/m³) and higher and females that received doses of 0.5 mg/m³ (2.0 μmol/m³) and higher had a significantly increased incidence of squamous epiglottis metaplasia (5/10 2-mg males [p ≤ 0.05] and 10/10 8-, 20-, and 50-mg males vs. 0/10 controls [p = 0.01]; 4/10 0.5- and 2-mg females [p ≤ 0.05], 10/10 8-, 20-, and 50-mg females vs. 0/10 controls [p ≤ 0.01]).</p> <p>Lungs: Positive (for metaplasia; males only) Males that received 50 mg/m³ (202 μmol/m³) had a significantly increased incidence of squamous metaplasia (5/10 vs. 0/10 controls, p < 0.05).</p> <p>Nasal Tissue: Positive (for cytoplasmic alteration) Males and females that received doses of 8 mg/m³ (32 μmol/m³) and greater had a significantly increased incidence of cytoplasmic alteration in the respiratory epithelium, characterized by the presence of brightly eosinophilic cytoplasmic inclusions within mucosal epithelial cells (8/10, 10/10, and 10/10 males, in order of increasing dose, vs. 0/10 controls [p ≤ 0.01]; 6/10, 10/10, and 10/10 females, in order of increasing dose, vs. 0/10 controls [p ≤ 0.01]).</p> <p>Based on the results of this bioassay and on previously published genetic toxicity, carcinogenicity, and toxicokinetic data, NTP concluded that 1-NP Ahas a high likelihood of being carcinogenic to the respiratory tract, particularly under exposure conditions that lead to significant accumulations of 1-NP in the lungs, and perhaps other</p>	Chan (1996)

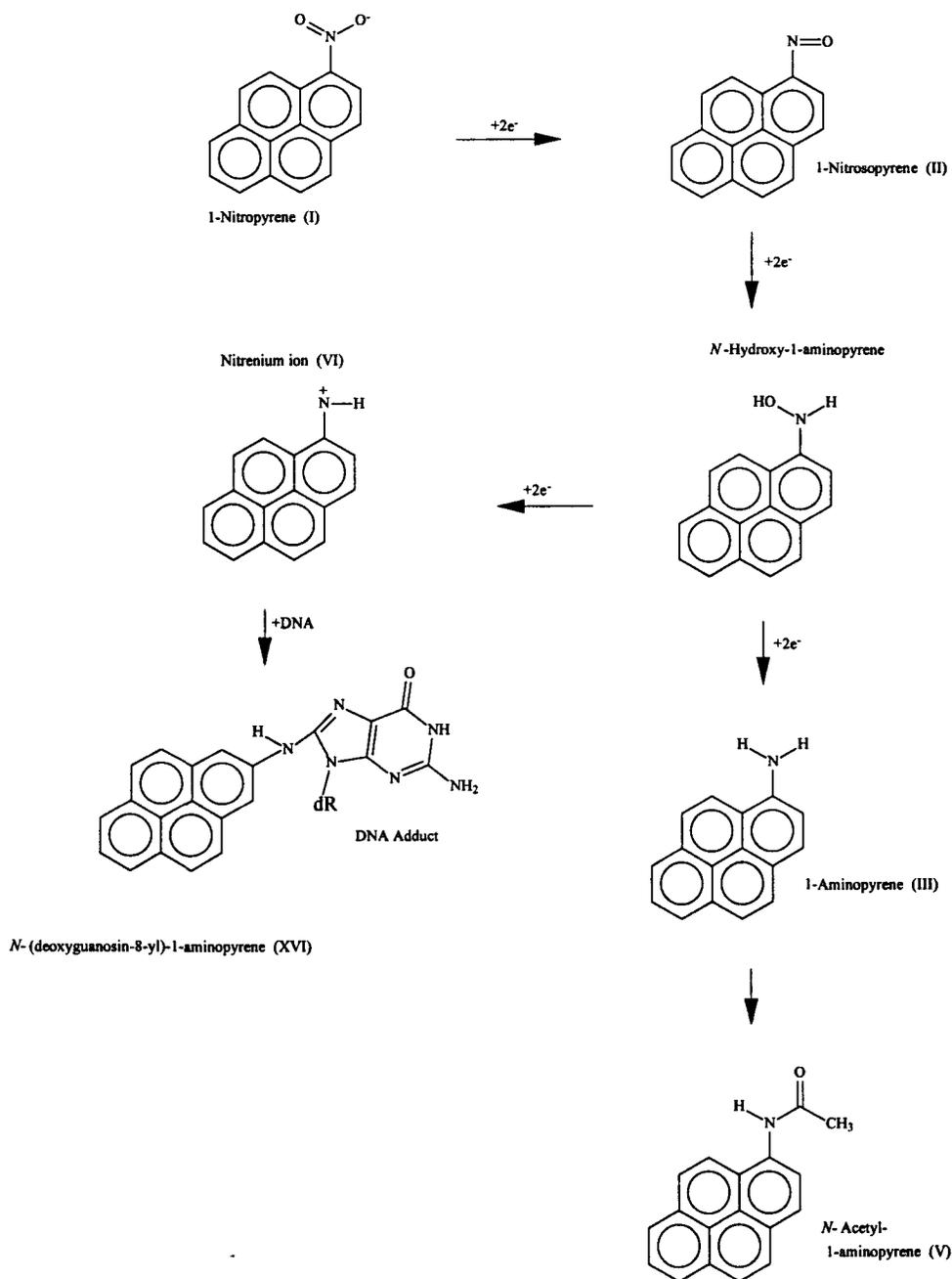
Table 6-2. Cell Proliferation Induced by 1-Nitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
						organs of F344/N rats.	
30-day-old CD rats	29F	29F (DMSO alone)	1-NP, purity not specified	67 µmol/kg bw in DMSO, 3 times/wk	4 wk	<p>Animals were killed 61 weeks after the first injection.</p> <p>Liver: Negative</p> <p>None of the 1-NP-treated rats displayed hyperplastic nodules or altered foci in liver. None of the rats developed hepatocellular carcinoma.</p>	Imaida et al. (1991a)
Hamsters - Intratracheal Instillation							
7- to 9-wk-old Syrian golden hamsters	<p>1) 55M (2mg [8.09 µmol], once/wk)</p> <p>2) 55M (1 mg [4.04 µmol], once/wk)</p> <p>3) 58M (2 mg [8.09 µmol], twice/wk)</p> <p>4) 60M (1 mg [4.04 µmol], twice/wk)</p>	<p>20M (no treatment; shelf control)</p> <p>50M (sterile saline alone)</p> <p>50M (carbon particles suspended in saline)</p> <p>50M (B[a]P; positive control)</p>	1-NP, 99.9% pure	1 or 2 mg (4 or 8 µmol) adsorbed onto an equal mass of carbon carrier particles (Stokes diameter 2-5 µm, greater than 70%), once or twice/wk, administered intratracheally	92 wk	<p>Hamsters that died or were found moribund during the treatment period were immediately necropsied. The study was terminated after 92 weeks of treatment, at which time all surviving hamsters were necropsied. The larynx, trachea, and other tissues showing gross abnormalities were examined. The incidences of hyperplasia were not statistically analyzed for significance.</p> <p>Trachea: Tracheal hyperplasia was detected in all hamsters (13/54, 15/53, 33/53, and 36/58 animals in groups 1, 2, 3, and 4, respectively, vs. 2/20 shelf controls, 17/49 saline controls, 34/48 particle controls, and 34/48 positive controls).</p> <p>Lung: Pulmonary hyperplasia was detected in all hamsters except shelf controls (29/55, 27/54, 36/56, and 27/58 animals in groups 1, 2, 3, and 4, respectively, vs. 8/50 saline controls, 23/48 particle controls, and 21/49 positive controls).</p> <p>Bronchiolar hyperplasia was detected in all hamsters (31/55, 30/54, 32/56, and 28/58 animals in groups 1, 2, 3, and 4, respectively, vs. 1/20 shelf controls, 4/50 saline controls, 16/48 particle controls, and 28/49 positive controls).</p>	Moon et al. (1990)

Table 6-2. Cell Proliferation Induced by 1-Nitropyrene (Continued)

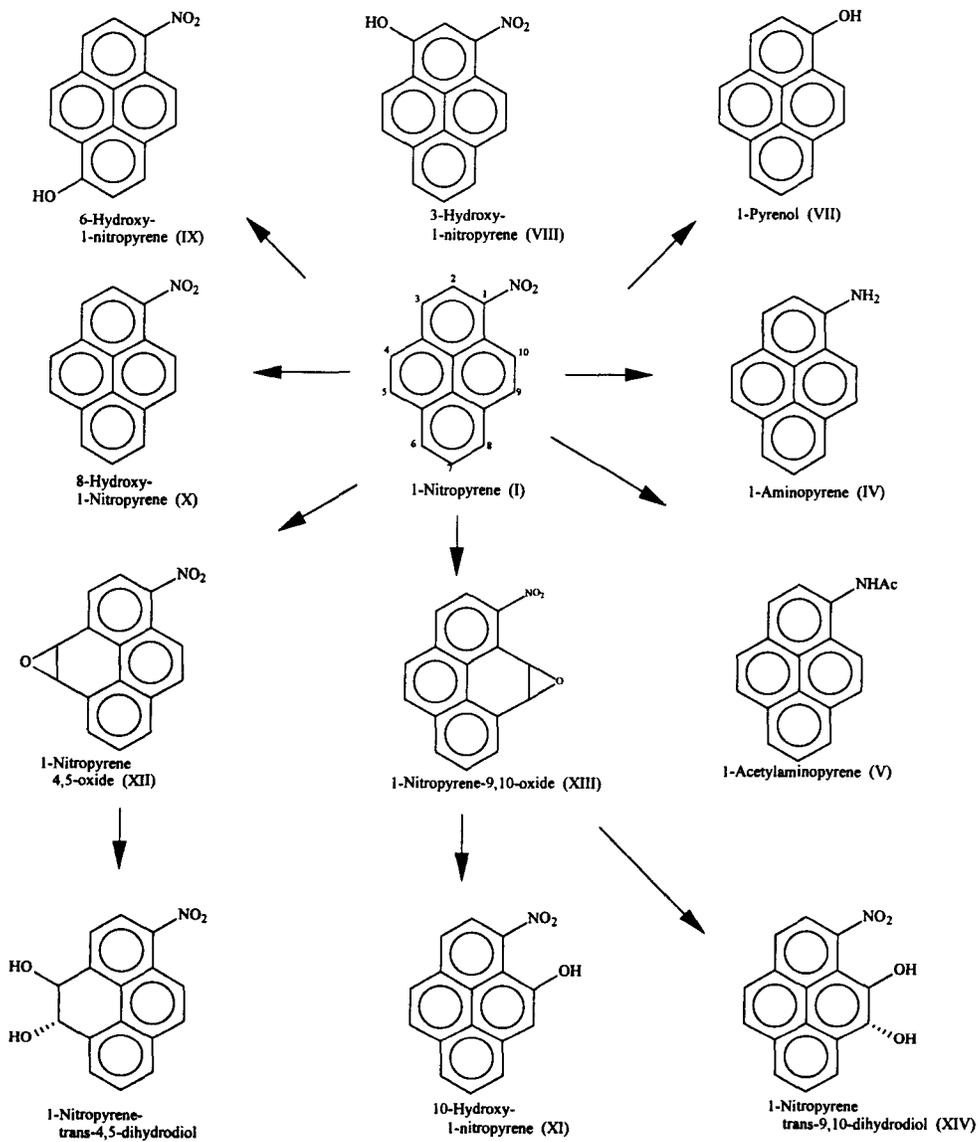
Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
<i>Mice - Dermal Application</i>							
7-wk-old SENCAR mice	6 groups of 39-40M and 6 groups of 39-40F	Positive controls: 40M, 40F Positive controls received a single dermal application of 0.05 mg B[a]P.	1-NP, > 99.5% pure	0-3.0 mg (0-12 µmol; other doses not specified) in 0.2 mL acetone, applied dermally in a single dose, except for highest dose, which was applied twice. Lower doses were not specified. Interval between high-dose applications was not specified.	Single or double application	One week after treatment, all mice received skin applications of 12-O-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL acetone, 2 times/wk for 30 weeks. Mice were evaluated at the end of this 30-week period. The statistical test used to analyze tumor incidence was not specified by IARC. Skin: Negative There was no significant increase in the incidence (not given) of skin papillomas in 1-NP-treated mice. All positive controls that survived past 31 weeks developed skin papillomas.	Nesnow et al. (1984; cited by IARC, 1989)
50- to 55-day-old CD-1 Charles River mice	20F	20F (acetone alone)	1-NP, > 99% pure	0.1 mg/0.1 mL acetone (4 µmol/mL), every other day, applied on shaved dorsal skin	20 days	Ten days after termination of 1-NP treatment, all mice received applications of 2.5 µg TPA in 0.1 mL acetone, 3 times/wk for 25 weeks. Mice were evaluated at the end of this 25-week period. The statistical test used to analyze tumor incidence was not specified by IARC. Skin: Negative The incidence of skin tumors (mainly papillomas) was not significantly increased in 1-NP-treated mice. IARC noted the small number of animals used.	El Bayoumy et al. (1982; cited by IARC, 1989)

Figure 6-1. Nitro Reduction Pathway for the Cellular Activation of 1-Nitropyrene in Mammalian Cells



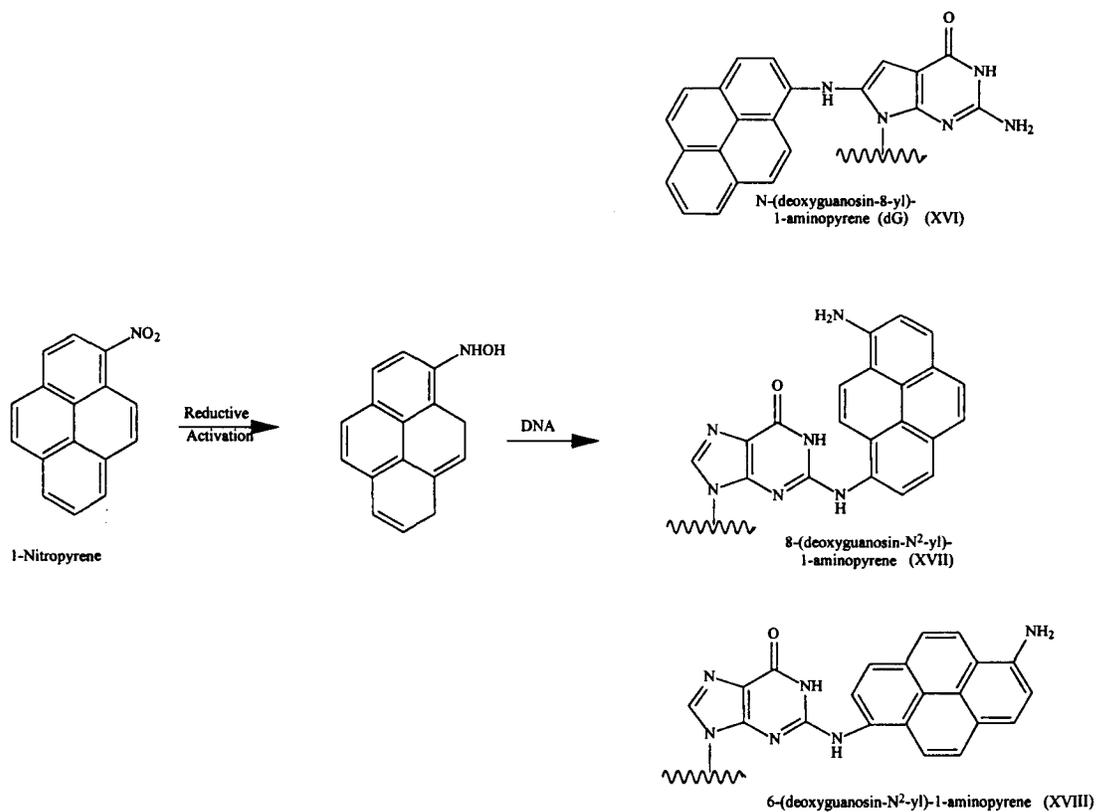
Source: Edwards et al. (1986; adapted from Patton et al., 1986)

Figure 6-2. Ring Oxidation Pathway for the Cellular Activation of 1-Nitropyrene in Mammalian Cells *In Vitro*



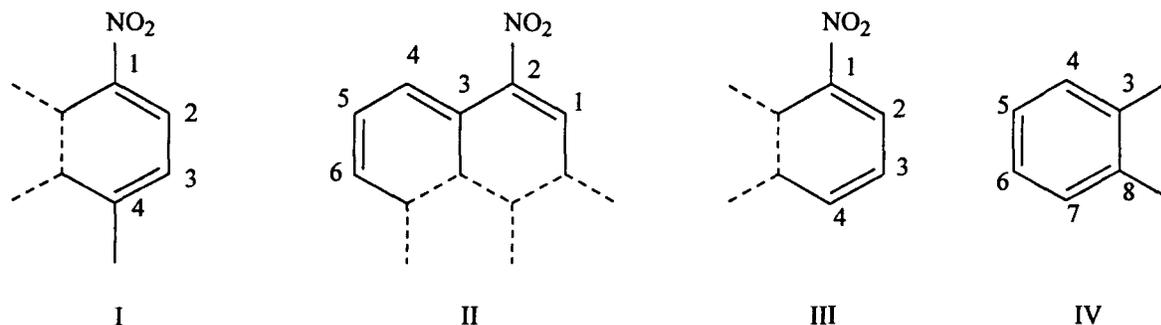
Source: Beland (1991)

Figure 6-3. 1-Nitropyrene DNA Adducts



Source: NTP (1996)

Figure 6-4. Fragments Responsible for the Mutagenicity of Nitroarenes



Fragments I and II are required for activity while fragments III and IV are deactivating. I differs from III in that C-4 is not bonded to a hydrogen (Klopman and Rosenkranz, 1984).

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES

**DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES
(IARC Monograph in Vol. 46, 1989)**

Online searching was done by the technical support contractor in TOXLINE January 30, 1996, using the CASRNs of the title compounds and *o*-nitroanisole and specifying publications after 1988. IARC (1989) was to be relied on for identification of pertinent earlier references. The 1240 records in TOXLINE were reduced by combining with the controlled vocabulary terms for metabolism and neoplasms and with the free-text truncated terms carcinogen? or mechanis? or toxicokinetic? or metab? From the 418 resulting records, the contractor selected approximately 160 for acquisition. Of the approximately 100 citations related to biological activity independently selected by the primary reviewer from NIEHS Review Group 1, 20 were identified as abstracts for which full publications were available; 73 had also been selected by the contractor. Thus, the primary reviewer selected 7 additional references that had not been identified as potentially useful by the contractor.

An exhaustive search of other pertinent toxicology databases was not attempted for the nitroarenes. A high degree of redundancy had been noted between TOXLINE and the databases CANCERLIT, EMBASE (Excerpta Medica), MEDLINE, and NIOSHTIC (Occupational Safety and Health). No special attempt was made to find toxicity information about metabolites and other structural analogues in the search strategy.

The contractor also searched CSCHEM and CSCORP for U.S. suppliers (Chem Sources databases); EMIC; EMICBACK; HSDB; IRIS; TSCATS (Toxic Substances Control Act Test Submissions); the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System), ISHOW (for physical-chemical properties), and REGMAT (May 1993 version; this regulatory information database with broad coverage of EPA regulations is no longer available); Chemical Abstracts Service's (CAS) CA and Registry Files for metabolism studies (152 records) and metabolite identification; CAS File CHEMLIST for TSCA and SARA updates in 1996; and CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information. For current awareness, the contractor monitored Current Contents on Diskette® Life Sciences 1200 [journals] edition. Older literature that needed to be examined was identified from the reviews and original articles as they were acquired.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonias obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test

<u>Code</u>	<u>Definition</u>
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange