

**NTP REPORT ON CARCINOGENS BACKGROUND
DOCUMENT for 1,6-DINITROPYRENE and
1,8-DINITROPYRENE**

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NTP Report on Carcinogens Listing for 1,6-Dinitropyrene

Carcinogenicity

1,6-Dinitropyrene 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).

When administered by subcutaneous (s.c.) injections, 1,6-dinitropyrene induced injection-site sarcomas in male mice and male and female rats, and leukemia in female rats (Tokiwa et al., 1984, and Ohgaki et al., 1985; cited by IARC V. 46, 1989; Imaida et al., 1995). Intraperitoneal (i.p.) injections of 1,6-dinitropyrene caused an increased incidence of liver-cell tumors in male mice (Wislocki et al., 1986; cited by IARC V. 46, 1989) and induced sarcomas of the peritoneal cavity in female rats (Imaida et al., 1991). Squamous cell carcinomas of the lung were induced in male rats receiving 1,6-dinitropyrene by intrapulmonary injection in two studies (Maeda et al., 1986; cited by IARC V.46, 1989; Iwagawa et al., 1989). The incidences of myeloid leukemia and lung adenocarcinomas were significantly increased in male and female hamsters receiving 1,6-dinitropyrene by intratracheal instillation (Takayama et al., 1985; cited by IARC V.46, 1989). 1,6-Dinitropyrene was found positive for carcinoma of the pituitary gland in an oral study of short-term duration in rats (Imaida et al., 1991).

There are no adequate data available to evaluate the carcinogenicity of 1,6-dinitropyrene in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Intratracheal administration of 1,6-dinitropyrene to rats previously inoculated to de-epithelialized trachea with an immortalized bronchial cell line, caused tumors when the tracheas were then implanted s.c. into nude mice (Iizasa et al., 1993). 1,6-Dinitropyrene is genotoxic in a wide variety of assays in bacteria and mammalian cells including human cells. 1,6-Dinitropyrene also demonstrates evidence of cell transformation activity in vitro in rat tracheal epithelial cells. Metabolic pathways leading to mutagenic and clastogenic metabolites and DNA adducts of 1,6-dinitropyrene have been described (IARC V.46, 1989).

No data are available that would suggest that the mechanisms thought to account for tumor induction by 1,6-dinitropyrene in experimental animals would not also operate in humans.

NTP Proposed Report on Carcinogens Listing for 1,8-Dinitropyrene

Carcinogenicity

1,8-Dinitropyrene 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).

When administered by subcutaneous (s.c.) injections, 1,8-dinitropyrene induced injection- site sarcomas in male mice and male and female rats, and leukemia in female rats (Imaida et al., 1995; Ohgaki et al., 1984, 1985; cited by IARC V.46, 1989; Otofujii et al., 1987; cited by IARC V.46, 1989). Intraperitoneal (i.p.) injections of 1,8-dinitropyrene induced sarcomas of the peritoneal cavity, leukemia, and mammary adenocarcinoma in female rats (Imaida et al., 1991b; 1995). The incidences of mammary tumors, including adenocarcinomas, were increased in female rats receiving 1,8-dinitropyrene by gavage (Imaida et al., 1991b; IARC V.46, 1989).

There are no adequate data available to evaluate the carcinogenicity of 1,8-dinitropyrene in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

1,8-Dinitropyrene is genotoxic in a wide variety of assays in bacteria and mammalian cells demonstrating evidence of cell transformation activity *in vitro*, and metabolic pathways leading to mutagenic and clastogenic metabolites and DNA adducts have been described (IARC V.46, 1989).

No data are available that would suggest that the mechanisms thought to account for tumor induction of 1,8-dinitropyrene 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

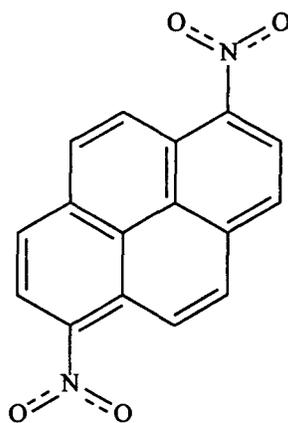
There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a 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 judgement, 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

1.1 Chemical Identification

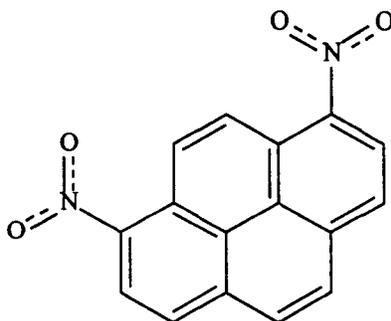
1,6-Dinitropyrene
[42397-64-8]



1,6-Dinitropyrene (C₁₆H₈N₂O₄, MW = 292.25 g/mol) is also called:

Pyrene, 1,6-dinitro-
1,6-DNP

1,8-Dinitropyrene
[42397-65-9]



1,8-Dinitropyrene (C₁₆H₈N₂O₄, MW = 292.25 g/mol) is also called:

Pyrene, 1,8-dinitro-
1,8-DNP

1.2 Physical-Chemical Properties

1,6-Dinitropyrene

Property	Information	Reference
Color	Yellow	Chemsyn Science Laboratories (1988; cited by IARC, 1989)
Physical State	Crystalline solid	Chemsyn Science Laboratories (1988; cited by IARC, 1989)
Melting Point, °C	310	Chemsyn Science Laboratories (1988; cited by IARC, 1989)
Solubility: Organic Solvents	Moderately soluble in toluene	Chemsyn Science Laboratories (1988; cited by IARC, 1989)

1,8-Dinitropyrene

Property	Information	Reference
Color	Yellow	Chemsyn Science Laboratories (1988; cited by IARC, 1989)
Physical State	Fluffy, crystalline solid	Chemsyn Science Laboratories (1988; cited by IARC, 1989)
Melting Point, °C	300	Chemsyn Science Laboratories (1988; cited by IARC, 1989)

1.3 Identification of Structural Analogues and Metabolites

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

- N*-Acetoxy-1-amino-6-nitropyrene (C₁₈H₁₂N₂O₄, MW = 320.30)
- N*-Acetoxy-1-amino-8-nitropyrene (C₁₈H₁₂N₂O₄, MW = 320.30)
- 1-Acetylamino-6-nitropyrene (1-Acetamido-6-aminopyrene, C₁₈H₁₂N₂O₃, MW = 304.30)
- 1-Acetylamino-8-nitropyrene (1-Acetamido-8-aminopyrene, C₁₈H₁₂N₂O₃, CASRN 99387-36-7, MW = 304.30)
- 1-Amino-6-nitropyrene (C₁₆H₁₀N₂O₂, CASRN 30269-01-3, MW = 262.27)
- 1-Amino-8-nitropyrene (C₁₆H₁₀N₂O₂, CASRN 30269-02-4, MW = 262.27)
- N*-Hydroxy-*N*-acetyl-1-amino-1,6- (or -1,8-)dinitropyrene (referred to as an arylhydroxamic acid), C₁₈H₁₂N₂O₄, MW = 320.30)
- N,N'*-Diacetyl-1,6-diaminopyrene (1,6-Diacetamidopyrene, C₂₀H₁₆N₂O₂, MW = 316.36)
- N,N'*-Diacetyl-1,8-diaminopyrene (1,8-Diacetamidopyrene, C₂₀H₁₆N₂O₂, MW = 316.36)
- 1,6-Diaminopyrene (C₁₆H₁₂N₂, MW = 232.28)
- 1,8-Diaminopyrene (C₁₆H₁₂N₂, CASRN 30269-04-6, MW = 232.28)
- N*-Hydroxy-1-amino-6-nitropyrene (1-NONH-6-NP, C₁₆H₁₀N₂O₃, MW = 278.27)
- N*-Hydroxy-1-amino-8-nitropyrene (1-NONH-8-NP, C₁₆H₁₀N₂O₃, MW = 278.27)
- 1-Nitro-6-nitrosopyrene (C₁₆H₈N₂O₃, MW = 276.25)
- 1-Nitro-8-nitrosopyrene (C₁₆H₈N₂O₃, MW = 276.25)
- 1-Nitroso-6-nitropyrene (1-NO-6-NP, C₁₆H₈N₂O₃, CASRN 101043-65-6, MW = 276.25)
- 1-Nitroso-8-nitropyrene (1-NO-8-NP, C₁₆H₈N₂O₃, CASRN 100593-23-5, MW = 276.25)
- Pyrenylnitrenium ion (C₁₆H₈N₂O₃, MW = 276.25)

Physical-chemical properties of the above structural analogues and metabolites could not be found. Structures for at least some of these analogues may be found in Figure 6-1.

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 Data, 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

There is no evidence that 1,6-dinitropyrene (1,6-DNP) has been used for other than laboratory applications. 1,6-DNP is available for research purposes at $\geq 98\%$ purity. It is also available in ^{14}C - or ^3H -labeled form at $\geq 98\%$ radiochemical purity (IARC, 1989).

1,8-Dinitropyrene (1,8-DNP) has been reported to be a photosensitizer, increasing the spectral activity of bis-azide compounds with light. However, there is no evidence that 1,8-DNP is currently used commercially for this or other applications. 1,8-DNP is available for research purposes at $\geq 98\%$ purity. It is also available in ^{14}C - or ^3H -labeled form at $\geq 98\%$ radiochemical purity (IARC, 1989).

2.2 Production

One U.S. company produces 1,6- and 1,8-DNP (SRI, 1992). No data on imports of 1,6- or 1,8-DNP were available (IARC, 1989). Chem Sources identified three U.S. suppliers of 1,6-DNP and four suppliers of 1,8-DNP (Chem Sources, 1996).

2.3 Exposure

2.3.1 Environmental Exposure

The primary route of potential human exposure to 1,6- and 1,8-DNP is inhalation. Detectable levels have been found in respirable particulates from ambient atmospheric samples. Higher amounts have been reported in heavy industrialized areas than in nonindustrialized urban and suburban sites. Both 1,6- and 1,8-DNPs have been found in extracts of particles from the exhaust of heavy-duty and light-duty diesel engines. They have also been found in small amounts in particulate emissions from kerosene heaters and gas burners. Prior to 1980, some carbon black samples known to be used in photocopy machines were found to contain considerable quantities of 1,6- and 1,8-DNPs (IARC, 1989). Both dinitropyrenes were detected in wastewater collected from oil-water separating tanks of 10 gasoline stations for 1 year (Manabe et al., 1984), and in exhaust particulates from idling gasoline and diesel engines. The total concentration of 1,6- and 1,8-DNP emitted from diesel engine vehicles was considerably higher than that of gasoline engine vehicles (Hayakawa et al., 1994).

Both 1,6- and 1,8-DNP biodegraded slowly in river water that had a low number of colony-forming units (CFU); in contrast, 1,6- and 1,8-DNP biodegraded rapidly in aerobic soil suspensions containing a high number of CFU, suggesting that "microflora in the environment play an important role in the primary degradation of and decontamination of relatively low concentrations of nitropyrenes" (Tahara et al., 1995).

1,6- and 1,8-DNP were not listed in the National Occupational Exposure Survey (1984) or the National Occupational Hazard Survey conducted (1986) by NIOSH.

2.3.2 Occupational Exposure

“Occupational exposures to PAHs can be measured, but samples are typically not large enough to allow quantitative measurements to be made” (IARC, 1989). Both 1,6- and 1,8-DNPs have been found in extracts of particles from the exhaust of heavy-duty and light-duty diesel engines, and IARC (1989) has listed several occupations that are associated with exhaust exposure from diesel engines, including railroad workers, mine workers, bus garage and other bus workers, truck drivers, fork-lift truck operators, and firefighters.

Scheepers et al. (1994a, 1994b, 1995) have also listed several other workplace environments that are associated with 1,6- and 1,8-DNP: River vessel (workplace), ship’s engine (source of diesel exhaust); Army driving lessons, armoured cars; flower auction, trucks; farming, tractor; gardening, passing traffic; airport platform, platform vehicles; concrete manufacturing, chemical plant, fork lift trucks; aluminum rolling, and galvanizing workshop, fork-lift trucks; grass verge maintenance, lawn mowers; and a river vessel, ships aggregate.

2.4 Regulations

OSHA regulates 1,6- and 1,8-DNP under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS^a

	Regulatory Action	Effect of Regulation/Other Comments
O S H A	29 CFR 1910.1200. Promulgated 2/15/89. OSH Act: Hazard Communication Standard.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2B), 1,6- and 1,8-DNP are included as chemical hazards in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan.

^a The regulations in this table have been updated through the 1996 Code of Federal Regulations: 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of 1,6- or 1,8-DNP in humans.

4.0 MAMMALIAN CARCINOGENICITY

Full details of mammalian carcinogenicity studies of 1,6- and 1,8-DNP are presented in Tables 4-1 and 4-2.

4.1 Mice

4.1.1 Subcutaneous Injection

The incidence of malignant fibrous histiocytomas at the injection site was significantly increased in 6-week-old male BALB/c mice treated with 0.342 μmol 1,6-DNP (Tokiwa et al., 1984; cited by IARC, 1989) or 0.170 μmol 1,8-DNP (Otofujii et al., 1987; cited by IARC, 1989) subcutaneously (s.c.) once per week for 20 weeks and observed for up to 60 weeks.

4.1.2 Intraperitoneal Injection

The incidence of liver tumors (adenomas and carcinomas) was significantly increased in newborn male CD-1 mice treated with a 0.2 μmol total dose of 1,6-DNP (administered as 3 intraperitoneal [i.p.] injections on days 1, 8, and 15 after birth) and killed after 1 year. Liver tumor incidence in females was not significantly increased compared to vehicle controls. Similar treatment with 1,8-DNP produced liver adenomas and carcinomas in males, but not females. The statistical significance of these tumors, however, was not specified (Wislocki et al., 1986; cited by IARC, 1989).

4.2 Rats

4.2.1 Oral Administration

The incidence of mammary tumors (adenocarcinoma, fibroadenoma, and adenoma) was significantly increased in weanling female CD rats treated with 10 μmol 1,8-DNP per kilogram mean body weight by gavage 3 times per week for 4 weeks. Similar treatment with 1,6-DNP did not induce a significant increase in the incidence of mammary tumors (King, 1988; Imaida et al., 1991b).

4.2.2 Subcutaneous Injection

The incidences of leukemia and malignant fibrous histiocytoma were significantly increased in newborn female CD rats treated with a total dose of 6.3 μmol 1,6- or 1,8-DNP s.c. over an 8-week period (week 1: 2.5 $\mu\text{mol}/\text{kg}/\text{wk}$, weeks 2-3: 5 $\mu\text{mol}/\text{kg}/\text{wk}$, weeks 4-8: 10 $\mu\text{mol}/\text{kg}/\text{wk}$) and observed for up to 67 weeks (King, 1988; Imaida et al., 1995). The incidence of tumors at other sites in these rats was not significantly increased.

The incidence of injection-site tumors was also significantly increased in 6-week-old male F344/DuCrj rats treated with 0.2 mg (0.7 μmol) 1,6-DNP or 0.002, 0.02, or 0.2 mg (0.007, 0.07, or 0.7 μmol) 1,8-DNP s.c. twice per week for 10 weeks (Ohgaki et al., 1984, 1985; both cited by IARC, 1989).

4.2.3 Intraperitoneal Injection

The incidences of malignant fibrous histiocytoma, leukemia, and mammary adenocarcinoma were significantly increased in weanling female CD rats treated with 10 μmol 1,8-DNP per kilogram mean body weight in DMSO (1.7 $\mu\text{mol}/\text{mL}$ DMSO) i.p. 3 times per week for 4 weeks and observed for up to 76-78 weeks. When another group of these rats was similarly treated with 1,6-DNP, there was a significant increase in the incidence of malignant fibrous histiocytoma, but not of any other tumor type (King, 1988; Imaida et al., 1991b).

4.2.4 Intrapulmonary Administration

The incidence of lung tumors (squamous cell carcinoma) was significantly increased in 10- to 11-week-old male F344/DuCrj rats 72 weeks after they received a single intrapulmonary injection of 0.05 mL beeswax-tricaprylin containing 0.513 μmol 1,6-DNP into the lower 1/3 of the left lung, following a left lateral thoracotomy (Maeda et al., 1986; cited by IARC, 1989).

In another study (Iwagawa et al., 1989), treatment of 11-week-old male F344/NSIc rats with an 0.05 mL suspension of 0.003, 0.01, 0.03, 0.1, or 0.15 mg (0.01-0.513 μmol) 1,6-DNP in equal volumes of beeswax and tricapyrin, following left lateral thoracotomy, induced squamous cell carcinoma of the lung in some rats after 104 weeks. Although none of the vehicle controls developed these tumors, the significance of the incidence in 1,6-DNP-treated rats was not specified.

4.2.5 Intratracheal Instillation

Iizasa et al. (1993) investigated the carcinogenic effects of intratracheal instillation of 1,6-DNP. An immortalized bronchial epithelial cell line, BEAS-2B, was inoculated into de-epithelialized F344 rat tracheas. Four weeks after inoculation, beeswax pellets containing 300 μg (1.03 μmol) 1,6-DNP were placed in the tracheal lumen. Controls received beeswax pellets without chemical. Six months following tracheal instillation, tracheal tumors were detected in 4/10 transplants exposed to 1,6-DNP and in none of the controls.

4.3 Hamsters

The incidence of myeloid leukemia was increased in ten-week-old male and female Syrian golden hamsters intratracheally administered 0.5 mg (1.7 μmol) 1,6-DNP suspended in 0.2 mL saline, once per week for 26 weeks (6/10 males, 6/10 females vs. none of controls; significance not specified) and observed for 11 months. All of the treated males and 9/10 treated females also developed lung adenocarcinomas. The increase was highly significant. Sixty-five percent of these animals had multiple tumor nodules (Takayama et al., 1985; cited by IARC, 1989). These data enhance the weight of evidence that 1,6-DNP is reasonably anticipated to be a human carcinogen because it represents a relevant route of human exposure.

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
<i>Mice - Subcutaneous Injection</i>							
6-wk-old BALB/c	20M	20M (DMSO alone)	1,6-DNP, >99.9% pure	0.1 mg (0.3 µmol) in 0.2 mL DMSO, once/wk	20 wk	<p>Mice were observed until moribund or up to 60 weeks. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Injection Site: Positive (for malignant fibrous histiocytoma)</p> <p>The first tumor in 1,6-DNP-treated mice was detected on day 112. Forty-five weeks after the first treatment, 10/20 1,6-DNP-treated mice (vs. none of the controls) had developed injection site tumors. These tumors were histologically diagnosed as malignant fibrous histiocytomas. IARC noted that this diagnosis is used to describe some subcutaneous and intraperitoneal sarcomas.</p> <p>Lung: Negative</p> <p>Lung tumors were detected in 6/20 1,6-DNP-treated mice and in 7/20 controls.</p>	Tokiwa et al. (1984; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - Intraperitoneal Injection							
newborn CD-1	groups of 90 or 100 M and F	groups of 90 or 100 M and F (DMSO alone) groups of 90 or 100 M and F (DMSO alone; started 10 weeks after first control group) groups of 90 or 100 M and F (positive control; 0.560 µmol B[a]P)	1,6-DNP, >99% pure	total dose: 0.2 µmol in 10, 20, or 40 µL DMSO, administered as 3 i.p. injections: on days 1, 8, and 15 after birth	15 days	All surviving mice were killed after 1 year. The statistical test used to analyze tumor incidence was not specified by IARC. Liver: Positive (for tumorigenesis in males) 1,6-DNP-treated males had a significantly increased incidence of liver tumors (adenoma: 3/25, carcinoma: 5/25) as compared to controls in group 1 (adenoma: 2/28 males in control group 1, 5/45 males in control group 2; no carcinoma detected). Lung: Negative There was no significant increase in the incidence of lung tumors in 1,6-DNP-treated males or females as compared to vehicle controls. Lymphatic System: Negative There was no significant increase in the incidence of malignant lymphoma in 1,6-DNP-treated males or females as compared to vehicle controls.	Wislocki et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity of 1,6-DNP (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Oral Administration							
weanling CD	36F	36F (DMSO alone)	1,6-DNP, >99% pure	10 µmol/kg bw in DMSO 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. There was no significant difference between the average survival time of 1,6-DNP-treated animals and of controls. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>Statistical analyses were performed using the χ^2 test and Student's <i>t</i>-test "wherever appropriate."</p> <p>Pituitary Gland: Positive (for carcinoma)</p> <p>There was an increase in the incidence of carcinoma of the pituitary gland in 1,6-DNP-treated rats (12/36 vs. 2/36 controls, p-value not given).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1991b)

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Subcutaneous Injection							
newborn CD	46F	40F (DMSO alone)	1,6-DNP, >99.9% pure	week 1: 2.5 $\mu\text{mol/kg}$ bw/wk weeks 2-3: 5 $\mu\text{mol/kg}$ bw/wk weeks 4-8: 10 $\mu\text{mol/kg}$ bw/wk 6.3 μmol average total dose/animal	8 wk	<p>Rats were killed when moribund or after 67 weeks. The average survival time of treated animals was not significantly different from that of controls. 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 analyses were performed using the χ^2 test.</p> <p>Blood: Positive (for leukemia)</p> <p>The incidence of leukemia was significantly increased in 1,6-DNP-treated rats (9/46 vs. 0/40 controls; $p < 0.005$)</p> <p>Injection Site: Positive (for malignant fibrous histiocytoma)</p> <p>All 1,6-DNP-treated rats (46/46) developed malignant fibrous histiocytoma (MFH), whereas none of the controls did ($p < 0.0001$).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1995)
6-wk-old F344/DuCrj	10M	20M (DMSO alone)	1,6-DNP, purity not specified, containing < 0.05% each of 1,3- and 1,8-DNP, 1,3,6-trinitropyrene, 1,3,6,8-tetranitropyrene	0.2 mg (0.7 μmol) in 0.2 mL DMSO, twice/wk	10 wk	<p>1,6-DNP-treated rats were killed on day 320. Controls were killed on day 650. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Injection Site: Positive (for sarcoma)</p> <p>All treated rats developed sarcomas at the injection site between days 103 and 123. None of the controls developed injection site tumors.</p>	Ohgaki et al. (1985; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene (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	36F	36F (DMSO alone)	1,6-DNP, >99% pure	10 µmol/kg bw in DMSO, 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>The average survival time of 1,6-DNP-treated rats was significantly decreased from that of controls (135 ± 13 days vs. 534 ± 48 days; p < 0.0001). The authors stated that the decreased survival of 1,6-DNP-treated rats was due to a high incidence of malignant fibrous histiocytoma (MFH) in these animals.</p> <p>Statistical analyses were performed using the χ^2 test and Student's <i>t</i>-test "wherever appropriate."</p> <p>Peritoneal Cavity: Positive (for MFH)</p> <p>All 1,6-DNP-treated rats (23/23) developed MFH, whereas none of the controls did (p < 0.0001).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1991b)
Rats - Intrapulmonary Administration							
10- to 11-wk-old F344/DuCrj	28M	31M (beeswax-tricaprylin alone) 19M (0.05 mL beeswax-tricaprylin + 0.5 mg methylcholanthrene)	1,6-DNP, >99.9% pure	After left lateral thoracotomy, rats received a single injection into the lower 1/3 of the left lung of 0.05 mL beeswax-tricaprylin containing 0.15 mg (0.513 µmol) 1,6-DNP	single dose	<p>Rats were observed for 72 weeks after treatment, at which time the study was terminated. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Lung: Positive (for squamous-cell carcinoma)</p> <p>Squamous-cell carcinoma was detected in 21/28 1,6-DNP-treated rats (vs. 0/31 vehicle controls). This tumor was also detected in all positive controls.</p>	Maeda et al. (1986; cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
11-wk-old F344/NSIc	39M (0.003 mg; 10 nmol) 30M (0.01 mg; 34.2 nmol) 31M (0.03 mg; 103 nmol) 26M (0.1 mg; 342 nmol) 9M (0.15 mg; 513 nmol)	40M (beeswax-tricaprylin alone) 29M, 30M, 29M, 13M (0.03, 0.1, 0.3, and 1.0 mg B[a]P, respectively)	1,6-DNP, 99.8% pure	0.003, 0.01, 0.03, 0.1, or 0.15 mg (0.01–0.51 µmol) as a suspension in equal volumes beeswax and tricaprylin A left lateral thoracotomy was performed and 0.05 mL suspension was injected directly into lower 1/3 of left lung.	single dose	<p>Rats were observed until moribund or up to 104 weeks after treatment, at which time the study was terminated. "Grossly apparent lesions and tissues from all major viscera were studied histologically."</p> <p>The mean survival time of rats that received the 3 highest doses was significantly decreased as compared to vehicle controls (82.2 weeks [p < 0.05], 72.9 weeks [p < 0.001], and 76.1 weeks [p < 0.01], in order of increasing dose, vs. 91 weeks for controls; Student's <i>t</i>-test).</p> <p>Lung: Positive (for tumorigenesis)</p> <p>The method of Peto et al. (1980; cited by Iwagawa et al., 1989) was used to calculate the significance of dose-response relationships of lung tumors (squamous cell carcinoma and undifferentiated neoplasms) induced by 1,6-DNP.</p> <p>There was a dose-response relationship for the induction of lung tumors by 1,6-DNP. The <i>z</i> values calculated by 'death rate' analysis, 'prevalence' analysis, and 'pooled' analysis were, respectively, 9.735 (p < 0.001, one-tailed), 3.352 (p < 0.001), and 23.006 (p < 0.001).</p> <p>Squamous cell carcinoma was only detected in rats that received the 3 highest doses of 1,6-DNP (2/31, 3/26, and 2/9, in order of increasing dose; p-value not given). Undifferentiated neoplasms were detected in all groups of 1,6-DNP-treated rats except the low-dose and vehicle control groups (4/30, 11/31, 19/26, and 4/9, in order of increasing dose; p-value not given).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in a number of other tissues.</p>	Iwagawa et al. (1989)

Table 4-1. Mammalian Carcinogenicity of 1,6-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Intratracheal Instillation							
?-wk-old F344	10 (sex not specified)	5 (sex not specified) Controls received beeswax pellets without chemical.	1,6-DNP, purity not specified	An immortalized bronchial epithelial cell line, BEAS-2B, was inoculated into de-epithelialized F344 rat tracheas. Four weeks after inoculation, beeswax pellets containing 300 µg (1.03 µmol) 1,6-DNP were placed in the tracheal lumen.	6 mo	Rats were observed for six months. Tracheas were examined histologically. Trachea: Positive (for tumorigenesis) Tumors were detected in 4/10 transplants exposed to 1,6-DNP (vs. none in controls). The significance of this was not specified.	Iizasa et al. (1993)
Hamsters - Intratracheal Instillation							
10-wk-old Syrian golden	10M, 10F	10M, 10F (saline alone)	1,6-DNP, >99.9% pure	0.5 mg (1.7 µmol) suspended in 0.2 mL saline, once/wk	26 wk	Hamsters were observed for 11 months. The statistical test used to analyze tumor incidence was not specified by IARC. Lung: Lung adenocarcinomas were detected in 10 males and 9 females treated with 1,6-DNP during weeks 20-48. Sixty-five percent of these animals had multiple tumor nodules. No mention was made of controls. Blood: Positive (for myeloid leukemia) Six treated males and 6 treated females, but none of the controls, developed myeloid leukemia.	Takayama et al. (1985; cited by IARC, 1989)

Abbreviations: B[a]P = benzo[a]pyrene; bw = body weight; DMSO = dimethyl sulfoxide; F = females; M = males; mo = months; wk = week(s)

Table 4-2. Mammalian Carcinogenicity of 1,8-Dinitropyrene

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	20M	20M (B[a]P; positive control) 20M (untreated)	1,8-DNP, >99.9% pure	0.05 mg (0.17 μ mol) in 0.2 mL DMSO, once/wk	20 wk	<p>Mice were observed until moribund or up to 60 weeks. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Injection Site: Positive (for tumorigenesis)</p> <p>The incidence of injection-site tumors in 1,8-DNP-treated mice was significantly increased as compared to controls (6/15 vs. none in controls). All subcutaneous tumors were diagnosed histologically as malignant fibrous histiocytomas. IARC noted that this diagnosis is used to describe some subcutaneous and intraperitoneal sarcomas.</p> <p>Lung and Liver: Some of the 1,8-DNP-treated mice developed lung and liver tumors, but tumor incidences were not given.</p>	Otofuji et al. (1987; cited by IARC, 1989)
Mice - Intraperitoneal Injection							
newborn CD-1	groups of 90 or 100 M and F	<p>groups of 90 or 100 M and F (DMSO alone)</p> <p>groups of 90 or 100 M and F (DMSO alone; started 10 weeks after first control group)</p> <p>groups of 90 or 100 M and F (positive control; 0.560 μmol B[a]P)</p>	1,8-DNP, >99% pure	total dose: 200 nmol in 10, 20, or 40 μ L DMSO, administered as 3 i.p. injections on days 1, 8, and 15 after birth	15 days	<p>All surviving mice were killed after 1 year. The statistical test used to analyze tumor incidence was not specified by IARC.</p> <p>Liver: Five of 31 1,8-DNP-treated males developed liver tumors (4 developed adenoma, 1 developed carcinoma). Two of 28 males in control group 1 and 5/45 male controls in group 2 developed liver adenomas. The significance of this was not specified.</p> <p>Lung and Lymphatic System: Negative</p> <p>There was no significant increase in the incidence of lung tumors or malignant lymphomas between 1,8-DNP-treated mice and controls.</p> <p>IARC noted the small number of animals per group and the short observation period.</p>	Wislocki et al. (1986; cited by IARC, 1989)

Table 4-2. Mammalian Carcinogenicity of 1,8-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Oral Administration							
weanling CD	36F	36F (DMSO alone)	1,8-DNP, >99% pure	10 µmol/kg bw in DMSO (1.7 nM DMSO) 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. There was no significant difference between the average survival time of 1,8-DNP-treated animals and of controls. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>Statistical analyses were performed using the χ^2 test and Student's <i>t</i>-test "wherever appropriate."</p> <p>Mammary Gland: Positive (for tumorigenesis)</p> <p>The total incidence of mammary tumors in 1,8-DNP-treated rats (adenocarcinoma, fibroadenoma, and adenoma) was significantly increased as compared to controls (22/36 vs. 2/36 controls, p-value not given).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1991b)

Table 4-2. Mammalian Carcinogenicity of 1,8-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Subcutaneous Injection							
newborn CD	37F	40F (DMSO alone)	1,8-DNP, >99.9% pure	<p><i>week 1:</i> 2.5 $\mu\text{mol/kg}$ bw/wk</p> <p><i>weeks 2-3:</i> 5 $\mu\text{mol/kg}$ bw/wk</p> <p><i>weeks 4-8:</i> 10 $\mu\text{mol/kg}$ bw/wk</p> <p>7.8 $\mu\text{mol/kg}$ bw/wk average total dose/animal</p>	8 wk	<p>Rats were killed when moribund or after 67 weeks. The average survival time of 1,8-DNP-treated rats was not significantly different from that of controls. 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 analyses were performed using the χ^2 test.</p> <p>Blood: Positive (for leukemia)</p> <p>The incidence of leukemia was significantly increased in 1,8-DNP-treated rats (8/37 vs. 0/40 controls; $p < 0.005$)</p> <p>Injection Site: Positive (for malignant fibrous histiocytoma)</p> <p>All 1,8-DNP-treated rats (37/37) developed malignant fibrous histiocytoma (MFH), whereas none of the controls did ($p < 0.0001$).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1995)

Table 4-2. Mammalian Carcinogenicity of 1,8-Dinitropyrene (Continued)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old F344/DuCj	10M (low dose) 10M (high dose)	20M (DMSO alone)	1,8-DNP, containing 0.4% 1,3-DNP	0.002 mg or 0.02 mg (0.007 or 0.07 μmol) in 0.2 mL DMSO, twice/wk	10 wk	1,8-DNP-treated rats were killed on day 320. Controls were killed on day 650. The statistical test used to analyze tumor incidence was not specified by IARC. Injection Site: Positive (for sarcoma) All rats treated with the high dose (10/10) developed sarcomas between days 123 and 156 and 9/10 rats treated with the low dose developed sarcomas between days 213 and 320 (vs. None of the controls).	Ohgaki et al. (1985; cited by IARC, 1989)
	10M		1,8-DNP, containing 0.4% 1,3-DNP and 0.05% other nitropyrenes	0.2 mg (0.7 μmol) in 0.2 mL DMSO, twice/wk		1,8-DNP-treated rats were killed on days 140 and 169. The statistical test used to analyze tumor incidence was not specified by IARC. Injection Site: Positive (for tumorigenesis) All 1,8-DNP-treated animals (10/10) developed sarcomas between days 113 and 127 (vs. none of the controls)	

Table 4-2. Mammalian Carcinogenicity of 1,8-Dinitropyrene (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	36F	36F (DMSO alone)	1,8-DNP, >99% pure	10 µmol/kg bw in DMSO (1.7 nM DMSO), 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. The average survival time of 1,8-DNP-treated rats was significantly decreased from that of controls (236 ± 54 days vs. 534 ± 48 days; p < 0.0001). The authors stated that the decreased survival of 1,8-DNP-treated rats was due to a high incidence of malignant fibrous histiocytoma (MFH) in these animals. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>Statistical analyses were performed using the χ^2 test and Student's <i>t</i>-test "wherever appropriate."</p> <p>Injection Site: Positive (for MFH)</p> <p>The incidence of MFH was significantly increased in 1,8-DNP-treated rats (29/33 vs. 0/31 controls; p < 0.0001).</p> <p>Blood: Positive (for leukemia)</p> <p>The incidence of leukemia was significantly increased in 1,8-DNP-treated rats (7/33 vs. 0/31 controls; p < 0.01).</p> <p>Mammary Gland: Positive (for adenocarcinoma)</p> <p>The incidence of mammary adenocarcinoma was significantly increased in 1,8-DNP-treated rats (14/33 vs. 3/31 controls; p < 0.001).</p> <p>Other: Negative</p> <p>There was no significant increase in the incidence of tumors in other tissues.</p>	King (1988); Imaida et al. (1991b)

Abbreviations: B[a]P = benzo[a]pyrene; bw = body weight; DMSO = dimethyl sulfoxide; F = females; M = males; mo = months; wk = week(s)

5.0 GENOTOXICITY

5.1 Genotoxicity of 1,6-Dinitropyrene

Studies of the genotoxic effects of 1,6-DNP are summarized in Table 5-1.

Summary: 1,6-DNP 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* in the absence of S9, 1,6-DNP was found to induce DNA damage in *Bacillus subtilis*, *Salmonella typhimurium*, mouse hepatocytes, and Chinese hamster V79 cells; unscheduled DNA synthesis (UDS) in mouse, rat, and hamster hepatocytes, rabbit lung Clara cells, and human tracheal/bronchial epithelial cells; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells; gene mutations in *Escherichia coli*, *S. typhimurium*, and CHO cells; chromosomal aberrations in rat and Chinese hamster liver epithelial cells, CHO cells, and human fibroblasts; micronuclei in rat hepatoma cells; and cell transformation in rat tracheal epithelial cells. It was negative only for DNA damage and DNA-protein crosslinks in rat hepatoma cells, gene mutations in human hepatoma cells, and micronuclei induction in mouse and human hepatoma cells. *In vivo*, it was negative for UDS in rat hepatocytes and spermatocytes. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

Information for studies reviewed in IARC was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided. In addition, for simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system were discussed as a group rather than cited individually. Because of the extensive information available in IARC (1989) on the *in vitro* and *in vivo* genotoxicity of 1,6-DNP, the post-1989 genotoxicity literature selection strategy was limited solely to studies that might offer additional unique information.

5.1.1 Noneukaryotic Systems

5.1.1.1 DNA Damage

1,6-DNP induced DNA damage in *B. subtilis* [LED = 0.02 µg/disc (0.07 nmol/disc)] (Horikawa et al. 1986; cited by IARC, 1989) and *S. typhimurium* [LED = 0.005 µg/disc (0.02 nmol/disc)] (Nakamura et al. 1987; cited by IARC, 1989) in the absence of metabolic activation.

5.1.1.2 Gene Mutations

Tokiwa et al. (1986; cited by IARC, 1989) reported that 1,6-DNP gave positive results for gene mutations in *E. coli* strain WP₂uvrA both with and without S9 activation [LED=0.0012 µg/plate (0.004 nmol/plate)].

IARC (1989) also reported that 1,6-DNP [LED = 0.00012 µg/plate (0.0004 nmol/plate)] gave positive results for gene mutations in various strains of *S. typhimurium* (TA96, TA97, TA98, TA100, TA102, TA104, TA1537, and TA1538) both with and without S9 activation (14 various authors cited by IARC, 1989). Oda et al. (1992) reported that 0.0003 to 0.01 µg/mL 1,6-DNP (0.001 to 0.03 µM) induced a 1.7-fold higher level of *lacZ* gene mutations in *S. typhimurium* strain NM1011, containing plasmids with both a nitroreductase gene and the *umuC-lacZ* fusion gene, than the parent untransformed strain. Recently, Busby et al. (1994) observed that 1,6-DNP exposure (0.0003 to 0.004 µg/mL; 0.009 to 0.014 µM) for 2 hours produced an approximately 150-fold increase in 8-azaguanine-resistant mutations in *S. typhimurium* strain

TM677 without S9 activation and an approximately 100-fold increase in mutations at 100-fold higher doses with S9 activation.

5.1.2 Lower Eukaryotic Systems

IARC (1989) reported on conflicting studies stating that 1,6-DNP either did [LED = 1.6 µg/mL (5.5 µM)] (Wilcox and Parry, 1981; Wilcox et al., 1982; both cited by IARC, 1989) or did not [HID = 500 µg/mL (1710 µM)] (McCoy et al. 1983; cited by IARC, 1989) induce an increase in gene conversions in the yeast *Saccharomyces cerevisiae*.

5.1.3 Mammalian Systems In Vitro

5.1.3.1 DNA Damage

1,6-DNP induced DNA damage, as measured by alkaline elution, in mouse hepatocytes [LED = 5.8 µg/mL (20 µM)] (Möller and Thorgeirsson, 1985; cited by IARC, 1989) and Chinese hamster V79 cells [LED = 4.4 µg/mL (15 µM)] (Saito et al. 1984; cited by IARC, 1989) without S9, but not in rat hepatoma cells [HID = 10 µM)] (Möller and Thorgeirsson, 1985; cited by IARC, 1989). The latter study also reported that 15µM 1,6-DNP did not induce DNA-protein cross-links in rat hepatoma cells.

IARC (1989) further reported that 1,6-DNP was found to give positive results for induction of UDS in primary rat and mouse hepatocytes [LED = 0.01 µg/mL (0.03 µM)] (Butterworth et al., 1983; Mori et al. 1987; both cited by IARC, 1989), rabbit lung Clara cells [LED = 0.63 µg/mL (2.2 µM)] (Haugen et al., 1986; cited by IARC, 1989), human hepatocytes [LED = 0.015 µg/mL (0.051 µM)] (Butterworth et al., 1983; cited by IARC, 1989), and tracheal or bronchial epithelial cells [LED = 0.015 µg/mL (0.051 µM)] (Doolittle et al. 1985; cited by IARC, 1989).

Edgar and Brooker (1985; cited by IARC, 1989) reported that 1,6-DNP gave positive results for the induction of SCE in CHO cells tested in the absence of S9 metabolic activation [LED = 0.05 µg/mL (0.2 µM)].

5.1.3.2 Gene Mutations

1,6-DNP induced a positive mutagenic response at the *hgpert* locus in CHO cells with and without S9 [LED = 0.05 µg/mL (0.2 µM)] (Edgar and Brooker, 1985; cited by IARC, 1989), V79 cells without S9 [LED = 0.1 µg/mL (0.3 µM)] (Kato et al., 1984; cited by IARC, 1989), and lung cells without S9 [LED = 0.57 µg/mL (2.0 µM)] (Nakayasu et al., 1982; cited by IARC, 1989), but not in human hepatoma cells at the *hprt* locus [HID = 2 µg/mL (7 µM)] (Eddy et al., 1985; cited by IARC, 1989).

5.1.3.3 Chromosomal Damage

1,6-DNP was found to give positive results for the induction of chromosome aberrations in rat liver epithelial cells [LED = 0.01 µg/mL (0.03 µM)] (Danford et al., 1982; cited by IARC, 1989) and CHO cells [LED = 0.05 µg/mL (0.2 µM)] (Edgar and Booker, 1985; cited by IARC, 1989) as well as human fibroblasts [LED = 0.02 µg/mL (0.07 µM)] (Wilcox et al., 1982; cited by IARC, 1989) in the absence of S9 activation. Adams et al. (1989) found that 0.1 to 10 µg/mL (0.34 to 34 µM) 1,6-DNP for 24 hours altered the mitotic and spindle morphology of human fibroblast cell line MRC5VA. Significant increases in condensed chromatin and chromosome clustering were seen at doses of 5.0 µg/mL (17 µM) and above. Spindle length was significantly

shortened at doses of 0.5 µg/mL (1.7 µM) and above. Roscher and Wiebel (1992) found that 1,6-DNP exposure for 24 hours induced micronuclei in rat hepatoma (0.3 to 10.0 µM; LED = 1.0 µM) as well as Chinese hamster lung V79 cells (0.01 to 0.1 µM; LED = 0.03 µM) in the absence of S9 activation, but not in mouse or human hepatoma cells (3.0 µM). They further reported that 1,6-DNP induced kinetochore positive micronuclei in the V79 cell line.

5.1.3.4 Cell Transformation

West and Rowland (1994) reported that 1,6-DNP at 0.15 to 1.5 µg/mL (0.51 to 5.1 µM) for 24 hours induced morphological transformations in male Fischer 344 rat tracheal epithelial cells. The highest tested dose increased the transforming frequency approximately 6-fold over the controls.

5.1.4 Mammalian Systems *In Vivo*

1,6-DNP did not induce an increase in SCE over controls in either hepatocytes or spermatocytes of rats (strain not provided) [LED = 50 mg/kg bw (0.17 mmol/kg bw) via gavage] (Butterworth et al., 1983; Butterworth, 1984; both cited by IARC, 1989).

5.2 Genotoxicity of 1,8-Dinitropyrene

Studies of the genotoxic effects of 1,8-DNP are summarized in Table 5-2.

Summary: 1,8-DNP was also found to exhibit reproducible genotoxicity in a wide variety of prokaryotic and mammalian *in vitro* and *in vivo* test systems [see Genetic Activity Profile, Figure 5-2 (Data limited to IARC, 1989)]. When tested *in vitro* in the absence of S9, 1,8-DNP was found to induce DNA damage in *Bacillus subtilis*, *Salmonella typhimurium*, mouse hepatocytes, rat hepatoma cells, and Chinese hamster V79 cells; unscheduled DNA synthesis (UDS) in mouse and rat hepatocytes and rabbit lung Clara cells; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells; gene mutations in *Escherichia coli*, *S. typhimurium*, mouse lymphoma, and CHO cells; chromosomal aberrations in rat epithelial cells, CHO cells, and human fibroblasts; and cell transformation in Syrian hamster embryo cells. It was negative only for DNA-protein crosslinks in rat hepatoma cells, UDS and gene mutations in human hepatoma cells, and micronuclei induction in human XP cells. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

Information for studies reviewed in IARC (1989) was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided. In addition, for simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system were discussed as a group rather than cited individually. Because of the extensive information available in IARC (1989) on the *in vitro* and *in vivo* genotoxicity of 1,8-DNP, the post-1989 genotoxicity literature selection strategy was limited solely to studies that might offer additional unique information.

5.2.1 Noneukaryotic Systems

5.2.1.1 DNA Damage

1,8-DNP induced DNA damage in *B. subtilis* [LED = 0.01 µg/disc (0.03 nmol/disc)] (Horikawa et al. 1986; cited by IARC, 1989) and *S. typhimurium* [LED = 0.003 µg/plate (0.01 nmol/plate)] (Nakamura et al. 1987; cited by IARC, 1989) in the absence of metabolic activation.

5.2.1.2 Gene Mutations

As reported by McCoy et al. (1984; cited by IARC, 1989), 1,8-DNP [LED = 0.125 µg/plate (0.428 nmol/plate)] was found to give positive results for gene mutations in *E. coli* strain WP₂uvrA without S9 activation.

IARC (1989) also reported that 1,8-DNP [LED = 0.0001 µg/plate (0.3 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 (14 authors cited by IARC, 1989). Oda et al. (1992) reported that 0.0001 to 0.01 µg/mL (0.3 to 30 µM) 1,8-DNP induced a 1.4-fold higher level of *lacZ* gene mutations in *S. typhimurium* strain NM1011, containing plasmids with both a nitroreductase gene and the *umuC-lacZ* fusion gene, than the parent untransformed strain. Most recently, Busby et al. (1994) observed that 1,8-DNP exposure for 2 hours produced an approximately 150-fold increase in 8-azaguanine-resistant mutations in *S. typhimurium* strain TM677 without S9 activation (0.25 to 4.0 ng/mL; 0.86 to 14 nM) and an approximately 100-fold increase with S9 activation (0.25 to 2.5 µg/mL; 0.86 to 8.6 µM). Jurado et al. (1994) found that 0.01 to 0.2 nmol 1,8-DNP/plate induced forward arabinose-resistant mutants in *S. typhimurium* strains BA14, BA14NR, BA14DNP, BA16, BA16NR, and BA16DNP. Positive responses were observed in the nitroreductase-deficient NR strains (LED = 0.01 nmol/plate) but not the acetyltransferase deficient DNP strains.

5.2.2 Lower Eukaryotic Systems

IARC (1989) reported conflicting studies stating that 1,8-DNP either did [LED = 1.6 µg/mL (5.5 µM)] (Wilcox and Parry, 1981; Wilcox et al., 1982; both cited by IARC, 1989) or did not [HID = 500 µg/mL (1710 µM)] (McCoy et al., 1983; cited by IARC, 1989) induce an increase in gene conversions in the yeast *Saccharomyces cerevisiae*.

5.2.3 Mammalian Systems *In Vitro*

5.2.3.1 DNA Damage

As compiled by IARC (1989), 1,8-DNP induced DNA damage, as measured by alkaline elution, in mouse hepatocytes [LED = 1.5 µg/mL (5.1 µM)] (Möller and Thorgeirsson, 1985; cited by IARC, 1989), Chinese hamster V79 cells [LED = 4.4 µg/mL (15 µM)] (Saito et al., 1984; cited by IARC, 1989) without S9, and rat hepatoma cells [LED = 1.5 µg/mL (5.1 µM)] (Möller and Thorgeirsson, 1985; cited by IARC, 1989). IARC (1989) also reported that 4.4 µg/mL (15 µM) 1,8-DNP (Möller and Thorgeirsson, 1985; cited by IARC, 1989) did not induce DNA-protein crosslinks in rat hepatoma cells.

IARC (1989) further reported that 1,8-DNP was found to give positive results for induction of UDS in primary rat and mouse hepatocytes [LED = 0.1 µg/mL (0.3 µM)] (Mori et al., 1987; cited by IARC, 1989), rabbit lung Clara and alveolar cells [LED = 0.63 µg/mL (0.55 µM)] (Haugen et al., 1986; cited by IARC, 1989), but not human hepatoma cells [HID = 2.0 µg/mL (6.8 µM)] (Eddy et al., 1985, 1986; cited by IARC, 1989).

Edgar and Brooker (1985; cited by IARC, 1989) reported that 1,8-DNP gave positive results for the induction of SCE in CHO cells tested in the absence of S9 metabolic activation [LED = 0.05 µg/mL (0.2 µM)].

5.2.3.2 Gene Mutations

1,8-DNP reportedly induced a positive mutagenic response at the *hprt* locus in CHO cells and the ouabain locus in V79 cells [LED = 0.05 µg/mL (0.2 µM)] (Edgar and Brooker, 1985; Takayama et al., 1983; both cited by IARC, 1989), and lung cells [LED = 0.75 µg/mL (2.6 µM)] (Nakayasu et al., 1982; cited by IARC, 1989), all in both the presence and absence of metabolic activation, but not in human hepatoma cells [locus not provided, HID = 2.0 µg/mL (6.8 µM)] (Eddy et al., 1985 abstract, 1986; cited by IARC, 1989). A positive response was also observed at the *tk* locus in mouse lymphoma L5178Y cells [LED = 0.1 µg/mL (0.3 µM)] (Edgar, 1985; cited by IARC, 1989).

5.2.3.3 Chromosomal Damage

As compiled by IARC (1989), 1,8-DNP was found to give positive results for the induction of chromosome aberrations in rat epithelial fibroblasts [LED = 0.01 µg/mL (0.03 µM)] (Wilcox et al., 1982; cited by IARC, 1989), CHO cells [LED = 0.05 µg/mL (0.2 µM)] (Edgar and Brooker, 1985; cited by IARC, 1989) as well as human fibroblasts [LED = 0.31 µg/mL (1.1 µM)] (Wilcox et al., 1982; cited by IARC, 1989) in the absence of S9. Arlett (1984; cited by IARC, 1989) further reported that 1,8-DNP exposure did not induce micronuclei in human xeroderma pigmentosum fibroblasts [HID = 2.5 µg/mL (8.6 µM)] in the absence of S9 activation.

5.2.3.4 Cell Transformation

Di Paolo et al. (1983; cited by IARC, 1989) found that 1,8-DNP induced morphological transformations in Syrian hamster embryo (SHE) cells [LED = 1.0 µg/mL (3.4 µM)].

5.2.4 Mammalian Systems *In Vivo*

As reviewed by IARC (1989), 1,8-DNP activated the c-Ki-*ras* oncogene in sarcoma cells isolated from rats (strain, dose levels, and route/length of exposure were not given).

5.3 Genotoxicity of 1,6 and 1,8-DNP Metabolites

Studies of the genotoxic effects of 1,6 and 1,8-DNP metabolites are summarized in Table 5-3.

Summary: Fifer et al. (1986a) observed that both 1,6-dinitrosopyrene (1,6-DNOP) and 1,8-dinitrosopyrene (1,8-DNOP) exposure at 2 to 4000 pmol/plate without S9 activation produced similar increases in *his* gene mutations in *S. typhimurium* strains TA98 and TA98NR but had much reduced activities in TA98/1,8-DNP. Overall, maximum mutagenic activities were only 7% (1,6-DNOP) and 8% (1,8-DNOP) that of their respective parent compounds.

Fifer et al. (1986a) also reported that both 1,6-DNOP and 1,8-DNOP at 2.5 to 10 µM in the absence of metabolic activation induced strong, dose-dependent increases in *hprt* gene mutations in CHO cells, greater than that of their respective parents compounds.

5.4 DNA Adducts

Summary: Analysis of lung and liver DNA 24 hours after a single i.p. dose of 1,6-DNP administered to male CD mice indicated the presence of one major DNA adduct *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene (Delclos et al., 1987; cited by IARC, 1989). Following exposure of 1,6-DNP to rats, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene (see

Table 6-1, Figure 6-1) was found in liver, mammary glands, peripheral blood lymphocytes, kidney, urinary bladder, and spleen lymphocytes, with lung tissue of male rats determined as the target tissue for DNA- adduct binding following 1,8-DNP exposure (Beland, 1986; Djurić et al., 1988; El-Bayoumy et al., 1994; Smith et al., 1995). Inactivation of nitroreduction by apigenin and tannic acid following *in vitro* exposure of 1,6-DNP to bacteria resulted in inhibition of mutagenesis. [Thus, activation of 1,6-DNP to the DNA-reactive intermediate involves nitroreduction]. In addition, incubations including rat liver cytosol and 1,6- or 1,8-DNP showed a 20- to 40-fold increase in DNA binding following the addition of AcCoA. Furthermore, the extent of AcCoA-dependent binding of DNP metabolites reflected the amount of nitroreduction as determined by aminonitropyrene formation (Djurić et al., 1985). Similar results were found by Orr et al. (1985; [for review, see Tokiwa and Ohnishi, 1985 and Djurić et al., 1988]) following the addition of AcCoA to incubations including 1,6- or 1,8-DNP and control rat liver cytosol, and in rats pretreated with 1-NP and subsequently i.p. administered 1,6-DNP. Tokiwa and Ohnishi (1985) found the binding of radiolabel to DNA was increased 20- to 40-fold after the addition of AcCoA to incubations, while Djurić et al. (1988) reported DNA binding in kidney was increased 1.6-fold following 1-NP pretreatment and subsequent 1,6-DNP administration, [suggesting induction of the nitroreductase pathway by pretreatment with 1-NP and increased binding of DNA adducts via the addition of AcCoA].

Table 5-1. Summary of 1,6-Dinitropyrene Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1.1 Noneukaryote Systems							
5.1.1.1 DNA Damage							
<i>Bacillus subtilis</i>	DNA damage	-	n.p.	0.02 - 0.06 mg/disc (0.07 - 0.21 nmol/disc)	positive	LED = 0.02 mg/disc (0.07 nmol/disc)	Horikawa et al. (1986; cited by IARC, 1989)
<i>Salmonella typhimurium</i> (strain not provided)	DNA damage	-	n.p.	n.g.	positive	LED = 0.005 mg/plate (0.02 nmol/plate)	Nakamura et al (1987; cited by IARC, 1989)
5.1.1.2 Gene Mutations							
<i>Escherichia coli</i> WP ₂ uvrA pKM101	<i>trp</i> ⁻ gene mutations	+/-	n.p.	n.g.	positive/ positive	LED = 0.0012 µg/plate (0.004 nmol/plate)	Tokiwa et al. (1986; cited by IARC, 1989)
<i>S. typhimurium</i> strains TA96, TA97, TA98, TA100, TA102, TA104, and TA1537	<i>his</i> gene mutations	+/-	n.p.	n.g.	positive/ positive	Results based on 8 strains, LED = 0.0002 µg/plate (0.0003 nmol/plate)	14 papers cited by IARC (1989)
<i>S. typhimurium</i> strain NM1011 containing plasmids with both a nitroreductase gene and the <i>umuC-lacZ</i> fusion gene	<i>lacZ</i> gene mutations (β-galactosidase expression)	-	n.p.	0.0003 to 0.01 µg/mL (0.9 to 30 µM)	positive	Strain NM1011 had a 1.7-fold higher β-galactosidase activity following 1,6-DNP treatment than the parent (untransformed) strain.	Oda et al. (1992)
<i>S. typhimurium</i> strain TM677	gene mutations (resistance to 8-azaguanine)	+/-	>99%	0.3 – 4.0 ng/mL (0.001 – 0.014 µM) for 2 hours	positive/ positive	1,6-DNP produced a ~150-fold increase without S9 and a ~100-fold increase with S9 over controls (LED = 1.0 ng/mL [3 nM] -S9, 0.5 ng/mL [2 nM] +S9).	Busby et al. (1994)
5.1.2 Lower Eukaryote Systems							
<i>Saccharomyces cerevisiae</i>	gene conversions	-	n.p.	1.6 - 500 µg/mL (5.5 - 1710 nmol)	mixed	Conflicting results in three studies; negative in 1 study (HID = 500 µg/mL [1710 nM]) or positive in 2 studies (LED = 1.6 µg/mL [5.5 mM])	3 papers cited by IARC (1989)

Table 5-1. Summary of 1,6-Dinitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1.3 Mammalian Systems <i>In Vitro</i>							
5.1.3.1 DNA Damage							
mouse hepatocytes	DNA damage (alkaline elution)	NA	n.p.	1.5 – 5.8 µg/mL (5 - 20 µM)	positive	LED = 20 µM	Möller and Thorgeirsson (1985; cited by IARC, 1989)
Chinese hamster V79 cells	DNA damage (alkaline elution)	-	n.p.	4.4 µg/mL (15 µM)	positive	None	Saito et al. (1984; cited by IARC, 1989)
rat hepatoma cells	DNA damage (alkaline elution)	NA	n.p.	1.5 – 2.9 µg/mL (5 - 10 µM)	negative	HID = 10 µM	Möller and Thorgeirsson (1985; cited by IARC, 1989)
rat hepatoma cells	DNA-protein crosslinks	NA	n.p.	4.4 µg/mL (15 µM)	negative	None	Möller and Thorgeirsson (1985; cited by IARC, 1989)
mouse & rat hepatocytes	unscheduled DNA synthesis (UDS)	NA	n.p.	0.01 - 10 mg/mL (0.03 - 34.2 µM)	positive	LED = 0.01 mg/mL (0.03 µM)	Butterworth et al. (1983) and Mori et al. (1987; both cited by IARC, 1989)
rabbit lung Clara cells	UDS	-	n.p.	0.63 - 10 µg/mL (2.2 - 34.2 µM)	positive	LED = 0.63 µg/mL (2.2 µM)	Haugen et al. (1986; cited by IARC, 1989)
human hepatocytes & tracheal or bronchial epithelial	UDS	NA	n.p.	0.0015 – 1.5 µg/mL (0.5 - 5.0 µM)	positive (hep. & epi.)	LED = 0.015 mg/mL (0.051 µM) for all three cell types	Butterworth et al. (1983) and Doolittle et al. (1985; both cited by IARC, 1989)
Chinese hamster ovary (CHO) cells	sister chromatid exchanges	-	n.p.	0.05 - 5 µg/mL (0.2 - 17 µM)	positive	LED = 0.05 µg/mL (0.2 µM)	Edgar and Brooker (1985; cited by IARC, 1989)

Table 5-1. Summary of 1,6-Dinitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1.3.2 Gene Mutations							
CHO, V79, and Chinese hamster lung (CHL) cells	<i>hprt</i> gene mutations	+/-	n.p.	(CHO) 0.005 - 5 µg/mL [0.017 - 17 mM]; (V79) only 0.1 µg/mL [0.3 nM]; (CHL) 0.1-10 µg/mL [0.34 - 34 mM]	positive/positive	LED = 0.05 µg/mL [0.2 mM] (CHO +/- S9), 0.1 µg/mL [0.3 mM] (V79 -S9 only), 0.57 µg/mL [2.0 mM] (lung -S9 only)	3 papers cited by IARC (1989)
human hepatoma cells	<i>hprt</i> gene mutations	-	n.p.	up to 2 µg/mL (7 µM)	negative	HID = 2 µg/mL (7 µM)	Eddy et al. (1985 abstract, 1986; cited by IARC, 1989)
5.1.3.3 Chromosomal Damage							
rat liver epithelial cells	chromosome aberrations	-	n.p.	0.01- 2.5 µg/mL (0.03 - 8.6 µM)	positive	LED = 0.01 µg/mL (0.03 µM)	Danford et al. (1982; cited by IARC, 1989)
Chinese hamster liver epithelial cells	chromosome aberrations	-	n.p.	2.0 mg/mL (6.8 mM)	positive	LED = 2.0 mg/mL (6.8 mM)	Danford et al. (1983 abstract; cited by IARC, 1989)
human fibroblasts	chromosome aberrations	-	n.p.	0.02 - 5.0 µg/mL (0.03 - 17 µM)	positive	LED = 0.02 µg/mL (0.07 µM)	Wilcox et al. (1982; cited by IARC, 1989)
CHO cells	chromosome aberrations	-	n.p.	0.05 - 5 µg/mL (0.2 - 17 µM)	positive	LED = 0.05 µg/mL (0.2 µM)	Edgar and Brooker (1985; cited by IARC, 1989)
human fibroblast cell line MRC5VA	mitotic and spindle morphology	-	n.p.	0.1 to 10 µg/mL (.34 to 34 µM) for 24 hours	positive	Significant increases in condensed chromatin and chromosome clustering seen at doses of 5.0 µg/mL (17 µM) and above. Spindle length significantly shortened at doses of 0.5 µg/mL (1.7 µM) and above.	Adams et al. (1989)
human hepatoma cells	micronuclei induction	NA	n.p.	3.0 µM for 24 hours	negative	500 cells per dose were counted. HID = 3.0 µM.	Roscher and Wiebel (1992)
mouse hepatoma cells	micronuclei induction	NA	n.p.	3.0 µM for 24 hours	negative	HID = 3.0 µM.	Roscher and Wiebel (1992)
rat hepatoma cells	micronuclei induction	NA	n.p.	0.3 to 10.0 µM for 24 hours	positive	LED = 1.0 µM.	Roscher and Wiebel (1992)

Table 5-1. Summary of 1,6-Dinitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
CHL V79 cells	micronuclei (+/- kinetochores) induction	-	n.p.	0.01 to 0.1 µM for 24 hours	positive	The LED for micronuclei induction was 0.03 µM while the LED for kinetochore positive micronuclei was 0.1 µM	Roscher and Wiebel (1992)
5.1.3.4 Cell Transformations							
male Fischer 344 rat tracheal epithelial cells	morphological transformation	-	>98%	0.15 to 1.5 µg/mL (0.5-5.1 µM) for 24 hours	positive	The LED, 1.5 µg/mL (5.1 µM), increased the transforming frequency ~6-fold over controls.	West and Rowland (1994)
5.1.4 Mammalian Systems <i>In Vivo</i>							
rats (strain not provided) hepatocytes/spermatocytes	UDS	NA	n.p.	50 mg/kg bw (0.17 mmol/kg bw) via gavage	negative (both cell types)	HID = 50 mg/kg bw (0.17 mmol/kg bw)	Butterworth et al (1983) and Working and Butterworth (1984; both cited by IARC, 1989)

Abbreviations: bw = body weight; HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided; UDS = unscheduled DNA synthesis

Table 5-2. Summary of 1,8-Dinitropyrene Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.2.1 Noneukaryote Systems							
5.2.1.1 DNA Damage							
<i>Bacillus subtilis</i>	DNA damage	-	n.p.	0.01 - 0.04 mg/disc (0.03 - 0.14 nmol/disc)	positive	LED = 0.01 mg/disc (0.03 nmol/disc)	Horikawa et al. (1986; cited by IARC, 1989)
<i>Salmonella typhimurium</i>	DNA damage	-	n.p.	n.g.	positive	LED = 0.003 mg/plate (0.01 nmol/plate)	Nakamura et al. (1987; cited by IARC, 1989)
5.2.1.2 Gene Mutations							
<i>Escherichia coli</i> WP ₂ <i>uvrA</i> pKM101	<i>trp</i> gene mutations	+/-	n.p.	n.g.	positive/ positive	LED = 0.0125 mg/plate (0.0428 nmol/plate)	McCoy et al. (1985; cited by IARC, 1989)
<i>S. typhimurium</i> strains TA96, TA97, TA98, TA100, TA102, TA104, TA1537, and TA1538	<i>his</i> gene mutations	+/-	n.p.	n.g.	positive/ positive	Results based on 8 strains, LED = 0.0001 mg/plate (0.0003 nmol/plate)	14 papers cited in IARC (1989)
<i>S. typhimurium</i> strain NM1011 containing plasmids with both a nitroreductase gene and the <i>umuC-lacZ</i> fusion gene	<i>lacZ</i> gene mutations (β-galactosidase expression)	-	n.p.	0.0001 to 0.01 μg/mL (0.3 to 30 nM)	positive	Strain NM1011 had a 1.4-fold higher β-galactosidase activity following 1,8-DNP treatment than the parent (untransformed) strain.	Oda et al. (1992)
<i>S. typhimurium</i> strain TM677	gene mutations (resistance to 8-azaguanine)	+/-	>99%	0.25 - 4.0 ng/mL [0.86 - 14 nM] (-S9) and 0.25 to 2.5 μg/mL [0.86 to 8.6 μM] (+S9) for 2 hours	positive/ positive	1,8-DNP produced a ~150-fold increase over controls without S9 and a ~100-fold increase over controls with S9 (LED = 0.5 ng/mL -S9, 0.75 μg/mL +S9).	Busby et al. (1994)
<i>S. typhimurium</i> strains BA14, BA14NR, BA14DNP, BA16, BA16NR, BA16DNP	forward <i>ara</i> (arabinose resistant) gene mutations	-	n.p.	0.01 to 0.2 nmol/plate	positive	Positive responses (LED = 0.01 nmol/plate) were observed in the nitroreductase-deficient NR strains but not the acetyltransferase-deficient DNP strains.	Jurado et al. (1994)

Table 5-2. Summary of 1,8-Dinitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.2.2 Lower Eukaryote Systems							
<i>Saccharomyces cerevisiae</i>	gene conversions	-	n.p.	1.6 - 500 µg/mL (5.5 - 1710 µM)	mixed	Conflicting results in 3 studies: negative in 1 study [HID = 500 µg/mL (1710 µM)] and positive in 2 studies [LED = 1.6 µg/mL (5.5 µM)]	Wilcox and Parry (1981) and McCoy et al. (1983; cited by IARC, 1989)
5.2.3 Mammalian Systems <i>In Vitro</i>							
5.2.3.1 DNA Damage							
Chinese hamster V79 cells	DNA damage (alkaline elution)	-	n.p.	4.4 µg/mL (15 µM)	positive	None	Saito et al. (1984; cited by IARC, 1989)
mouse hepatocytes	DNA damage (alkaline elution)	NA	n.p.	1.5 - 5.8 µg/mL (5 - 20 µM)	positive	LED = 1.5 µg/mL (5.1 µM)	Möller and Thorgeisson (1985; cited by IARC, 1989)
rat hepatoma cells	DNA damage (alkaline elution)	NA	n.p.	0.9 - 2.9 µg/mL (3 - 10 µM)	positive	LED = 1.5 µg/mL (5.1 µM)	Möller and Thorgeisson (1985; cited by IARC, 1989)
rat hepatoma cells	DNA-protein cross-links	NA	n.p.	4.4 µg/mL (15 µM)	negative	None	Möller and Thorgeisson (1985; cited by IARC, 1989)
human hepatoma cells	unscheduled DNA synthesis (UDS)	NA	n.p.	n.g.	negative	HID = 2.0 µg/mL (6.8 µM)	Eddy et al. (1985 abstract; cited by IARC, 1989)
mouse & rat hepatocytes	UDS	NA	n.p.	0.01 - 10 µg/mL (0.03 - 34 µM)	positive	LED = 0.1 µg/mL (0.3 µM)	Mori et al. (1987; cited by IARC, 1989)
rabbit lung Clara & aveolar cells	UDS	-	n.p.	0.63 - 10 µg/mL (2.2 - 34 µM)	positive	LED = 0.63 µg/mL (2.2 µM)	Haugen et al. (1986; cited by IARC, 1989)
Chinese hamster ovary (CHO) cells	sister chromatid exchanges	-	n.p.	n.g.	positive	LED = 0.05 µg/mL 0.2 µM)	Edgar and Brooker (1985; cited by IARC, 1989)

Table 5-2. Summary of 1,8-Dinitropyrene Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.2.3.2 Gene Mutations							
CHO, V79, and Chinese hamster lung (CHL) cells	<i>hprt</i> gene mutations	+/-	n.p.	0.05 - 5 µg/mL [0.17 - 17 µM] (CHO), 0.01 - 0.1 µg/mL [0.03 - 0.34 µM] (V79), 0.03 - 8.0 µg/mL [0.10 - 27 µM] (CHL)	positive/positive	CHO/V79 LED = 0.05 µg/mL (0.2 µM); CHL LED = 0.75 µg/mL (2.6 µM)	3 papers cited by IARC (1989)
human hepatoma cells	<i>hprt</i> gene mutations	NA	n.p.	n.g.	negative	HID = 2.0 µg/mL (6.8 µM)	Eddy et al. (1985, 1986; cited by IARC, 1989)
mouse lymphoma cells	<i>tk</i> gene mutations	-	n.p.	0.01 - 5 µg/mL (0.03 - 17 µM)	positive	LED = 0.1 µg/mL (0.3 µM)	Edgar (1985; cited by IARC, 1989)
5.2.3.3 Chromosomal Damage							
CHO fibroblasts	chromosome aberrations	-	n.p.	0.05 - 5.0 µg/mL (0.17 - 17 µM)	positive	LED = 0.05 µg/mL (0.2 µM)	Edgar and Brooker (1985; cited by IARC, 1989)
human fibroblasts	chromosome aberrations	-	n.p.	0.02 - 5.0 µg/mL (0.07 - 17 µM)	positive	LED = 0.31 µg/mL (1.1 µM)	Wilcox et al. (1982; cited by IARC, 1989)
rat epithelial fibroblasts	chromosome aberrations	-	n.p.	0.01 - 2.5 µg/mL (0.03 - 8.6 µM)	positive	LED = 0.04 µg/mL (0.1 µM)	Danford et al. (1982; cited by IARC, 1989)
human xeroderma pigmentosum fibroblasts	micronuclei induction	-	n.p.	n.g.	negative	HID = 2.5 µg/mL (8.6 µM)	Arlett et al. (1984); cited by IARC, 1989)
5.2.3.4 Cell Transformation							
Syrian hamster embryo cells	morphological transformation	NA	n.p.	n.g.	positive	LED = 1.0 µg/mL (3.4 µM)	Di Paolo et al. (1983; cited by IARC, 1989)

Abbreviations: bw = body weight, HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided; UDS = unscheduled DNA synthesis

Table 5-3. Summary of 1,6- and 1,8-Dinitropyrene Metabolite Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Chemical & Purity	Doses Used	Endpoint Response	Comments	Reference
5.3.1 Noneukaryote Systems							
5.3.1.1 Gene Mutations							
Auxotrophic strains of <i>Salmonella typhimurium</i> strains TA98, TA98NR, and TA98/1,8-DNP ₆	<i>his</i> gene mutations	-	1-Nitro-6-nitrosopyrene; purity n.p.	2 - 4000 pmol/plate	positive	Similar levels of mutagenicity in strains TA98 and TA98 but had a much reduced activity in strain TA98/1,8-DNP ₆ . Maximum mutagenic activity only 7% that of parent compound (see section 6.0 and relevant table 6-1).	Fifer et al. (1986a)
			1-Nitro-8-nitrosopyrene; purity n.p.			Similar levels of mutagenicity in strains TA98 and TA98 but had a much reduced activity in strain TA98/1,8-DNP ₆ . Maximum mutagenic activity only 8% that of parent compound.	
5.3.2 Mammalian Systems <i>In Vitro</i>							
Chinese hamster ovary cells	<i>hprt</i> locus mutations	-	1-Nitro-6-nitrosopyrene	2.5 - 20 µM	positive	Strong dose-dependent increase of 6-TG-resistant cells which was greater than that of its parent compound. [~20 6-TG -resistant mutants/10 ⁶ survivors (2.5 µM) to ~100 6-TG -resistant mutants/10 ⁶ survivors (20 µM)].	Fifer et al. (1986a)
			1-Nitro-8-nitrosopyrene			Strong dose-dependent increase which was greater than that of its parent compound. [~55 6-TG -resistant mutants/10 ⁶ survivors (2.5 µM) to ~120 6-TG -resistant mutants/10 ⁶ survivors (20 µM)].	

Table 5-4. Summary of 1,6- and 1,8-Dinitropyrene DNA Adducts

Test System	Biological Endpoint/DNA Adduct	S9 Metab. Activation	Chemical & Purity	Doses Used	Endpoint Response	Comments	Reference
5.4.1 Prokaryote Systems							
<i>Salmonella typhimurium</i> strain TA1538	<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-8-nitropyrene; <i>N</i> -(Deoxyguanosin-8-yl)-1-amino-6-nitropyrene	-	1,6- and 1,8-DNP; purities n.p.	0.05 and 3 µM	positive	Similar adduct formed when reduced intermediate <i>N</i> -hydroxy-1-amino-8-nitropyrene was reacted with DNA (Djurić et al., 1985; see section 6.0).	Djurić et al. (1986b); Heflich et al. (1985a); Andrews et al. (1986); Beland (1986)
5.4.2 Mammalian Systems <i>In Vitro</i>							
Rat liver cytosols in the presence of calf liver DNA	<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-6-nitropyrene	-	1,6-DNP; purity n.p.	0.32 µg	positive	Incubations including rat mammary cytosol and 1,6- or 1,8-DNP in the presence of AcCoA formed metabolite binding to tRNA.	Imaida et al. (1988)
5.4.3 Mammalian Systems <i>In Vivo</i>							
Rat liver, mammary glands, peripheral blood lymphocytes, kidney, and urinary bladder	<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-8-nitropyrene; <i>N</i> -(Deoxyguanosin-8-yl)-1-amino-6-nitropyrene	-	1,6- and 1,8-DNP; purities n.p.	0.1 to 1000 µg/kg bw	positive in all tissues	DNA adduct postulated to form via a substitution at C8 of dG with nitrenium ion intermediates. Radioactivity bound to heme moiety of hemoglobin.	Beland (1986); El-Bayoumy et al. (1994).
Rat and mouse (male) lung (target tissue), liver, WBC, and spleen lymphocytes (surrogate tissues)	<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-6-nitropyrene	-	1,6-DNP; purity n.p.	2.1 and 6.8 nM	positive	None	Delclos et al. (1987, cited by IARC, 1989); Smith et al. (1995).

Abbreviations: bw = body weight; n.p. = not provided

Figure 5-1. Genetic Activity Profile of 1,6-Dinitropyrene
(Data limited to IARC, 1989)

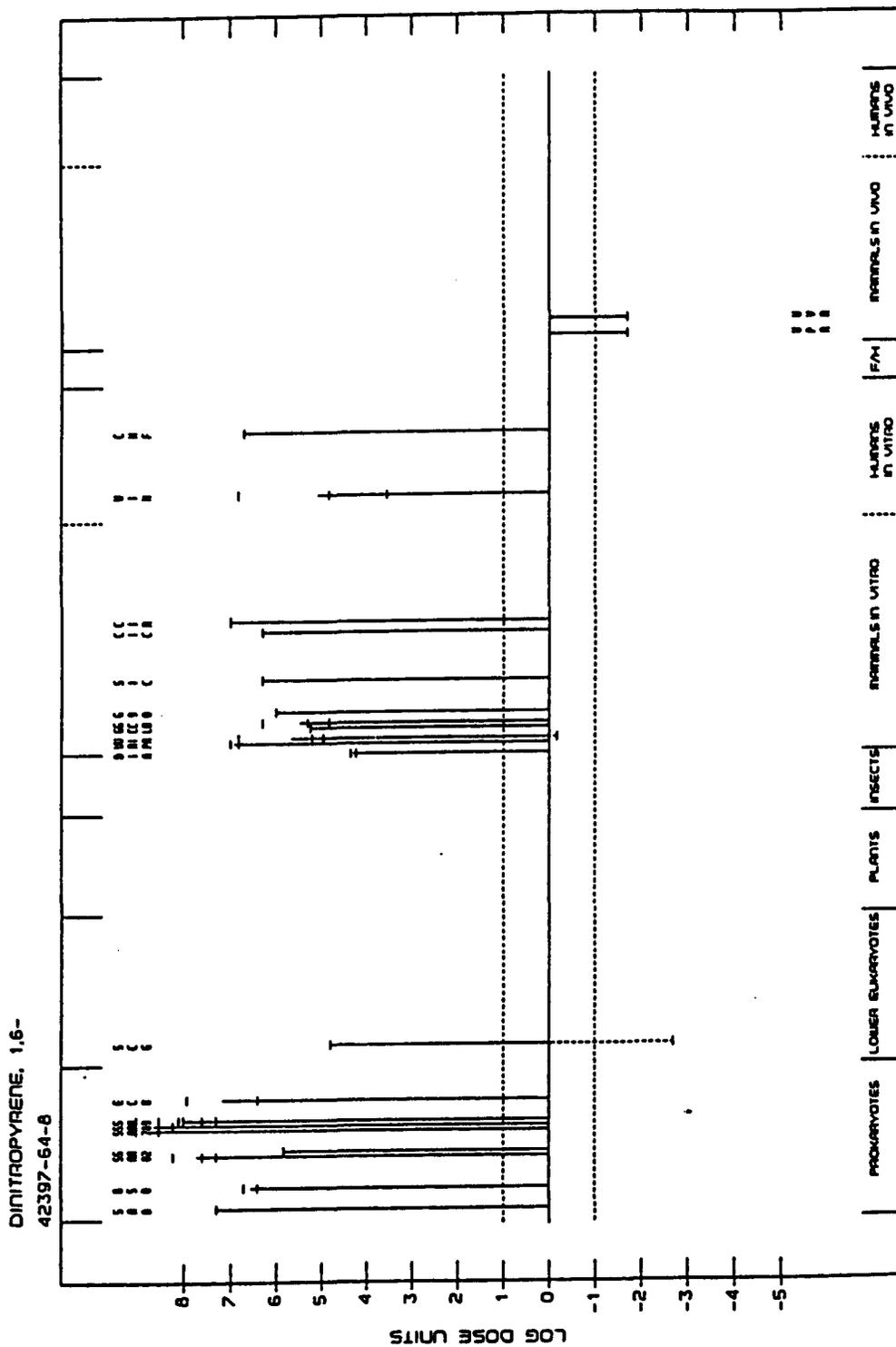


Figure 5-2. Genetic Activity Profile of 1,8-Dinitropyrene
(Data limited to IARC, 1989)

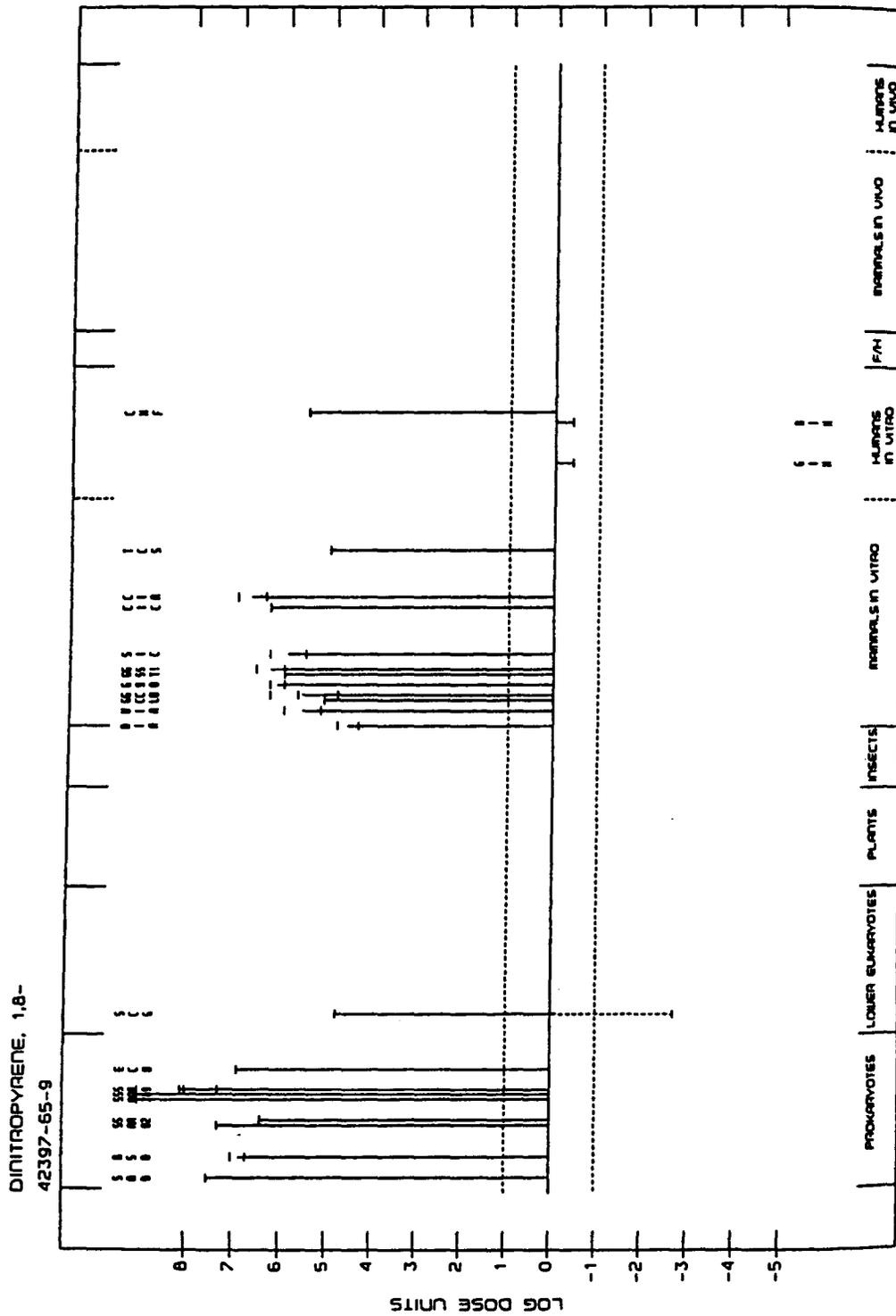
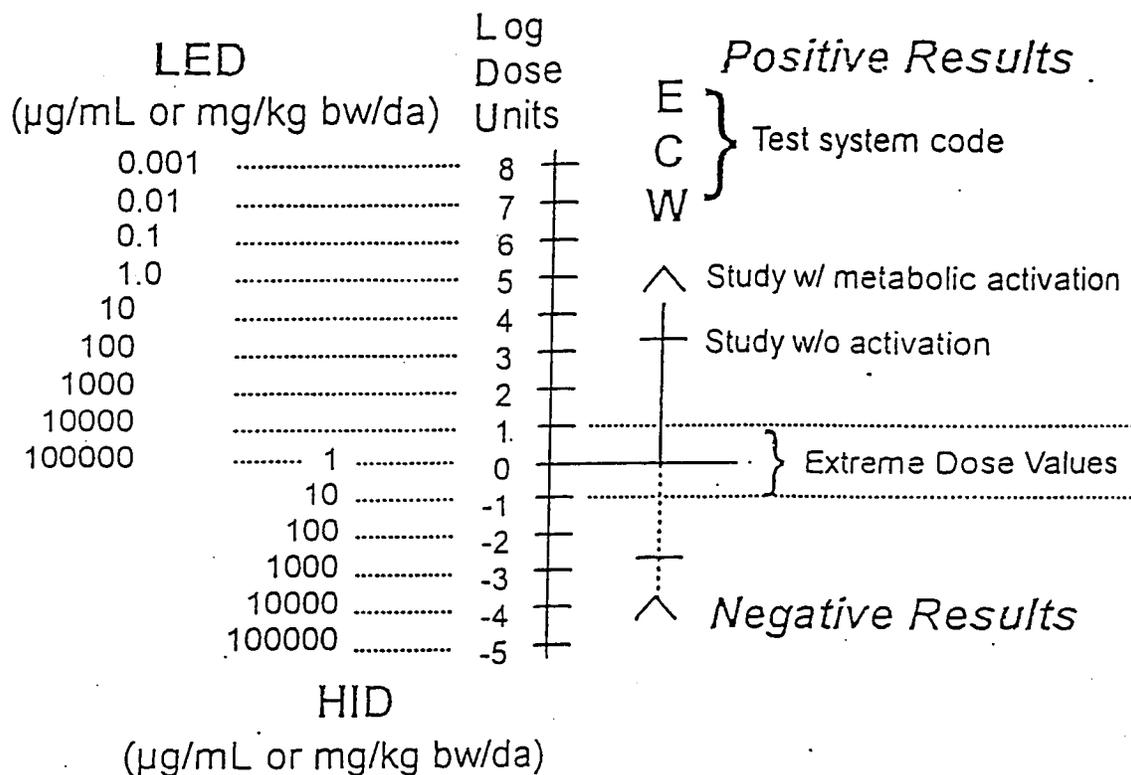


Figure 5-3. Schematic View of a Genetic Activity Profile (GAP)



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 DATA

6.1 Absorption, Distribution, Metabolism, and Excretion

6.1.1 Absorption, Distribution, and Excretion

Forty-eight hours after female Balb/c mice were orally dosed with [¹⁴C]1,8-DNP, 42% of the dose was eliminated in feces and 12% in urine. Within 9 days after dosing, fecal excretion was determined as the major excretory pathway, with 45% of the dose being eliminated by this route. Up to 6 h after administration of the dose, there was a linear increase in the concentration of radioactive material in the blood, liver, and kidneys, representing 0.27, 2.9, and 0.21% of the radioactive dose, respectively. The corresponding figures decreased to 0.1, 1.6, and 0.12% 24 hours after administration of the dose. Two, 4, and 6 hours after administration of the dose, >85% of radioactivity was detected in the gastrointestinal (g.i.) tract (stomach, small intestine, and large intestine), suggesting that 1,8-DNP is poorly absorbed by this route (Shah et al., 1990).

Shah et al. (1990) conducted studies using ligated sections of mouse g.i. tract to monitor the absorption of 1,8-DNP from small and large intestine and stomach. The overall rate of absorption of 1,8-DNP from the g.i. tract was poor, with ~81% of the dose recovered from the small intestine and ~19% recovered from the large intestine after 120 min following injection of the dose into the appropriate section of the g.i. tract. 1,8-DNP was not absorbed from the stomach (Shah et al., 1990).

Several nitroreduced metabolites of 1,8-DNP were identified in the feces of conventional male CD rats following oral administration: *N,N'*-diacetyl-1,8-diaminopyrene, 1-acetylamino-8-nitropyrene, and 1-amino-8-nitropyrene. In germ-free rats treated similarly, only one nitroreduced metabolite 1-amino-8-nitropyrene was identified in feces, suggesting the importance of intestinal microflora in the metabolism of 1,8-DNP (Heflich et al., 1986; cited by IARC, 1989).

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 the DNP metabolites are shown in the generic metabolic pathway depicted in Figure 6-1 (Proposed Mechanism for Activation of Nitrated Pyrenes to DNA Binding Species). Figure 6-1 has been postulated by Beland (1986) following *in vitro* incubations of rat and dog liver cytosols with 1,6- or 1,8-DNP in the presence of calf thymus DNA.

Both nitroreduction and AcCoA-dependent acetylation are involved in the metabolism of DNP to form the DNA adduct *N*-(deoxyguanosin-8-yl)-1-amino-6- or 8-nitropyrene (IX). 1,6- and 1,8-DNP (I) undergo sequential nitroreduction to form nitrosopyrenes (II) and *N*-hydroxylamines (III) which are further activated by AcCoA. Beland (1986) postulated that the AcCoA-dependent binding of DNP metabolites may occur through the formation of an arylhydroxamic acid (IV) which is capable of rearranging to a reactive *N*-acetoxyarylamine (V) via *N,O*-acetyltransferase. However, substitutions at the peri (C-1,8) positions with arylhydroxamic acids have been shown to be poor substrates for this enzyme (Flammang et al., 1985a,b; cited by Beland, 1986). Thus, direct AcCoA-dependent acetylation of the intermediate (III) to (V) was suggested as a more plausible pathway by Beland and confirmed by Fu et al. (1990). Subsequently, nitrenium ion intermediates (VI) are formed and have been postulated to bind to DNA. Further reduction of III can lead to the formation of 1-amino-6- or 8-nitropyrene (VII) and subsequent acetylation via AcCoA to *N*-acetylamino-6- or 8-nitropyrene (VII).

Human liver microsomal fractions catalyzed very little nitroreduction of I (< 4 pmol/mg protein/min) under aerobic and/or anaerobic conditions. In aerobic incubations, ring-oxidized

metabolites were not identified, whereas the addition of FMN to anaerobic incubations increased the nitroreduction of I to corresponding II (nitroso) and VII (amino) metabolites approximately 5-fold. Similar results have been reported by Shimada et al. (1990) following *in vitro* exposure of human and rat liver microsomes to 1,8-DNP.

Several studies have demonstrated the importance of intestinal microflora in the metabolism of DNP *in vivo* and *in vitro* (Cerniglia et al., 1986; cited by IARC, 1989; Heflich, 1986; Cerniglia et al., 1988; both cited by Rafii et al., 1991). Anaerobic bacterial suspensions from intestinal contents of Rhesus monkeys and rats and from human feces were reported to reduce 1,8-DNP to 1-amino-8-nitropyrene and 1,8-diaminopyrene (Cerniglia et al., 1986). Subsequently, Cerniglia et al. (1988; cited by Rafii et al., 1991) reported that intestinal microflora from human, Rhesus monkey, and rat metabolized 1,8-DNP to compounds that are less mutagenic than the parent compound. More recently, Rafii et al. (1991) identified the specific organisms responsible for detoxification of 1,6-DNP by anaerobic bacteria isolated from the human g.i. tract: *Clostridium paraputrificum*, *Clostridium perfringens*, and a *Eubacterium* species.

6.2 Modes of Action

There is some evidence that the activation of oncogenes plays a role in the carcinogenicity of 1,6- and 1,8-DNP. Thus, it is likely that one or more genotoxic events are steps in the induction of tumors by 1,6- and 1,8-DNP.

6.2.1 Oncogene Associations

Ochiai et al. (1985b) investigated the activation by 1,8-DNP of oncogenes in rat fibrosarcomas. Male F344 rats were injected with 0.2 mg (0.7 μ mol) 1,8-DNP dissolved in DMSO. By transfecting high molecular weight DNA extracted from the resulting fibrosarcomas into NIH 3T3 cells, the oncogenes in 7 fibrosarcomas were examined. Four of these sarcomas had transformants (number not specified) containing rat-specific repetitive DNA sequences. In a transformant from one of the four sarcomas and from 7 secondary transformants from the same sarcoma, *K-ras* sequences were identified. However, another transformant from this sarcoma did not have a *K-ras* sequence. Further examination showed that it also did not contain *H-ras*, *N-ras*, or *neu* sequences, implying that the parent sarcoma must have had at least two transforming genes (i.e., *K-ras* and another unknown gene). In the three other sarcomas with transformants, no *ras* or *neu* transforming genes were found.

IARC (1989), reviewing a study conducted by Ishizaka et al. (1987), reported that 1,6-DNP-induced rat fibrosarcomas contained activated *H-ras* and *N-ras* oncogenes. The incidence, species, dose, and route/length of exposure were not specified.

Neft et al. (1993) investigated whether a correlation existed between DNA adduct formation by 1,6- or 1,8-DNP and amplification of specific DNA sequences. C060 cells, derived from an SV40-transformed CHO cell line, were incubated for 5 h with 1.0 or 2.5 ng DNP/mL DMSO (0.0034 or 0.0085 nmol/mL DMSO). Incubation with 1,6-DNP produced the adduct *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene, while incubation with 1,8-DNP produced what was thought to be *N*-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene. Upon examination of DNA isolated from cells incubated with 1,6- or 1,8-DNP, it was determined that β -actin was not amplified and *c-fos* was only slightly amplified. The *c-fos* amplification reached a maximum increase of 5-fold, but it did not occur in a dose-response manner. However, SV40 DNA sequences in C060 cells were amplified by 1,6- and 1,8-DNP in a dose-response manner, with a

maximum increase of 15- to 20-fold at a dose of 2.5 ng DNP/mL DMSO (0.0085 nmol/mL DMSO).

6.2.2 Metabolism and Genotoxicity

The mutagenicity of 1,6- and 1,8-DNP in *S. typhimurium* (Djurić et al., 1986b; Heflich et al., 1985a; Andrews et al., 1986; Beland, 1986) and in mammalian systems may be ascribed to the ability of some of their metabolites to bind to DNA (Djurić et al., 1988; Imaida et al., 1988; Beland, 1986; El-Bayoumy et al., 1994; Smith et al., 1995). The activation route that produces these metabolites has been elucidated using *S. typhimurium* (see section 6.0, Table 6-1, and Figure 6-1) and human, rat, and dog liver cytosols (for review, see Table 6-1). It involves nitroreduction of I (Quilliam et al., 1982; Bryant et al., 1984; Djurić et al., 1985; Heflich et al., 1985; Orr et al., 1985; Tokiwa and Ohnishi, 1985; Fifer et al., 1986a,b; Tee et al., 1988; Beland, 1986; Djurić and McGunagle, 1989; Watanabe et al., 1989; Kuo et al., 1992; Shimada et al., 1990; Einistö et al., 1991; Hajos and Winston, 1991; Sawada et al., 1991; Neft et al., 1993; Perchermeier et al., 1994) to II (Djurić et al., 1986; Heflich et al., 1986; King et al., 1986; Beland, 1986; Beland et al., 1988; Djurić and McGunagle, 1989) via human P-450 IIA4, rat P-450 IA1 and IA2, and rabbit liver/lung NADPH-cytochrome P-450 reductase, xanthine oxidase, DT-diaphorase, and/or aldehyde oxidase (Tee et al., 1988). II may be further nitroreduced to *N*-hydroxylamino-mononitro intermediates III (Tokiwa and Ohnishi, 1985; Djurić et al., 1985; Beland, 1988; Beland et al., 1988, 1990; Djurić and McGunagle, 1989) via xanthine oxidase (Djurić et al., 1989) that are subsequently acetylated (Quilliam et al., 1982; McCoy et al., 1983; Bryant et al., 1984; Djurić et al., 1985; Heflich et al., 1985; Orr et al., 1985; Tokiwa and Ohnishi, 1985; Tokiwa et al., 1985; Fifer et al., 1986a&b; Beland, 1986; Djurić et al., 1988; Imaida et al., 1988; Tee et al., 1988; Djurić et al., 1989; O'Donovan, 1990; Srivastava and Wiebel, 1990; Watanabe et al., 1990; Einistö et al., 1991; Neft et al., 1993; Perchermeier et al., 1994; Silvers et al., 1994; Watanabe et al., 1994) to *N*-acetoxyaminonitropyrenes (V) that are catalyzed by human NAT1 and NAT2 *N*-acetyltransferase (Watanabe et al., 1994) and cytosolic *N,O*-acetyltransferase (AcCoA) (Tokiwa and Ohnishi et al., 1985; Djurić et al., 1985; Beland, 1986). AcCoA-dependent acetylation of the intermediate (III) to (V) was suggested as a more plausible pathway by Beland (1986) and confirmed by Fu et al. (1990). Subsequently, nitrenium ion intermediates (VI; postulated by Beland, 1986) are substituted at C-8 of deoxyguanosine, thereby forming the predominant DNA adduct IX (Beland, 1986; Imaida et al., 1988; Fu, 1990).

Several studies have been conducted using various strains of *S. typhimurium* and clearly show a correlation of nitroreduction and subsequent acetylation with mutations in these prokaryotes. For instance, after reviewing McCoy et al. (1983), Heflich et al. (1985), Orr et al., (1985), Fifer et al. (1986a,b), Watanabe et al. (1990), and Einistö et al. (1991), it was found that nitroreduction was the major metabolic pathway in *S. typhimurium*; however, increased nitroreductase activity of YG1021 and YG1026 did not result in increased mutagenicity when compared to their parent strains. Subsequent studies conducted by Fifer et al. (1986a) showed that the DNPs are metabolized to mutagens through nitroreduction, but their mutagenic activity is governed through the subsequent acetylation of the nitroreduced intermediates. [These findings explain the lack of increased mutagenicity in strains YG1021 and YG1026 (increased nitroreductase activity)]. Similar findings have been reported by McCoy et al. (1983), Orr et al. (1985), Fifer et al. (1986b), and Einistö et al. (1991), using the acetyltransferase-deficient strain TA98/1,8-DNP₆ and strains YG1024 and YG1029 (both have elevated *O*-acetyltransferase activity). In brief, the acetyltransferase-deficient strains showed a decrease in mutagenicity

following I exposure, while YG1024 and YG1029 showed a higher susceptibility to I mutagenicity than their parent strains, suggesting the importance of *O*-acetylation in the activation of I. Subsequent studies conducted by Djurić and McGunagle (1989) confirmed these findings; the authors stated that the mutagenicity of I in *S. typhimurium* was due to the latter's ability to acetylate the nitroreduced metabolites of I. The authors also stated that while *N*-acetylation may be a detoxification pathway (see Figure 6-1), *O*-acetylation of *N*-hydroxylamine derivatives enhances DNA binding. Confirming this hypothesis, using *S. typhimurium* with deficiency, or overproduction, of *O*-acetyltransferase, Jurado et al. (1994; see Table 5-2) found that bacteria defective in *O*-acetyltransferase (TA98DNP/BA14DNP and TA100DNP/BA16DNP) showed a diminished response in mutagenicity toward 1,8-DNP.

Recent *in vitro* studies, using CHO cells expressing human NAT1 or NAT2 *N*-acetyltransferase and a CHL cell line that stably expressed *O*-acetyltransferase of *S. typhimurium*, showed that the sensitivity to mutagenic effects of I was in the order NAT1 > NAT2 > parent CHL cells, suggesting that human NAT1 and NAT2 are involved in the metabolic activation of I to mutagenic species. In addition, CHL cells stably expressing *S. typhimurium* *O*-acetyltransferase were more sensitive than parent CHL cells were to mutagenic effects of I.

6.3 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 6-2).

6.4 Cell Proliferation

The incidence of renal hyperplasia was increased in weanling female CD rats administered 10 μmol 1,6- or 1,8-DNP per kilogram mean body weight intragastrically 3 times per week for 4 weeks (1,6-DNP: 12/36 vs. 5/36 controls; 1,8-DNP: 12/36 vs. 5/36 controls). In rats treated with 1,8-DNP, but not 1,6-DNP, there was also an increased incidence of altered foci in liver cells and of hyperplastic hepatic nodules (altered foci: 5/36 vs. 2/36 controls; hyperplastic nodules: 6/36 vs. 2/36 controls). When the rats were treated i.p. with the same dose (10 μmol 1,6- or 1,8-DNP per kilogram mean body weight 3 times per week for 4 weeks), there was no increase in proliferative activity in a number of tissues (King, 1988; Imaida et al., 1991b).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
Parent Compounds					
1,6-Dinitropyrene; 1,6-DNP [42397-64-8] (I)		Human NCI-H322 lung and liver HepG2 cell lines (Srivastava and Wiebel, 1990). CHL cells (Sawada et al., 1991).	<i>S. typhimurium</i> TA98 (Kuo et al., 1992)	Human Cytochrome P-450 _{BNF-B} and P-450 _{ISF-G} (Shimada and Guengrich, 1990). Acetyltransferase activation following nitroreduction (Perchermeier et al., 1994). DNP nitroreductase: NAD(P)H-quinone oxidoreductase (Hajos and Winston, 1991). Nitroreduction (McCoy et al., 1990) via cytochrome P-450 reductase (Sawada et al., 1991).	1,6-DNP was nitroreduced to a greater extent than 1,8-DNP (Hajos and Winston, 1991), and the only metabolites detected from I and II <i>in vitro</i> and <i>in vivo</i> have been those resulting from nitroreduction (Fu, 1990). DNP nitroreductase induction by Aroclor-1254, resulted in 15-fold increase in cytosolic NAD(P)H-quinone oxidoreductase activity (Hajos and Winston, 1991). I and II are activated by rat liver cytosol to DNA-binding species via nitroreduction to <i>N</i> -hydroxy aminonitropyrenes followed by <i>O</i> -acetylation of the <i>N</i> -hydroxy group (Fu, 1990). High acetyltransferase activity (120-270 nmol/min x mg protein) found in hamster (V79), rat hepatoma (H411EC3G ⁻) and fibroblast (208F) cell lines in comparison to human lung cells showing low N-acetylase activity (<15 nmol/min/mg protein; NCI-H322). Human cell lines were known to be insensitive (NCI-H322) or slightly sensitive (HepG2) to toxic effects of I compared to cell lines mentioned above, suggesting that acetylation is a major step for activation of I in some human cells (Srivastava and Wiebel, 1990). Similar results found by Silvers et al. (1994) (see I comments below).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
1,6-Dinitropyrene; 1,6-DNP (I) (contd.)		<p>Human and rat liver cytosol and microsomes (Fifer et al., 1986b; Beland, 1986; Djurić and McGunagle, 1989; see comments).</p> <p>Hamster H411EC3/G⁻ and rat BWI-J (Perchermeier et al., 1994).</p>	<p><i>Salmonella</i> S105 (Fifer et al., 1986b, Beland, 1986; see comments).</p> <p><i>S. typhimurium</i> TA98 and TM677 and rat liver S9 (Mermelstein et al., 1982; Shah et al., 1990, 1991; Busby et al., 1994).</p>	<p>Acetyltransferase activation following nitroreduction (Perchermeier et al., 1994).</p>	<p>I was highly toxic to the acetyltransferase-competent V79/NH and H4IIEC3/G cells but not to cytochrome P-450 competent rat hepatoma 5L or mouse BWI-J cells (Perchermeier et al., 1994) [suggesting that acetyltransferase is involved in the metabolic activation of I to a cytotoxic species].</p> <p>Fifer et al. (1986b), Beland (1986) Djurić and McGunagle (1989), Fifer et al. (1986b), and Tee et al. (1988) found that under aerobic conditions, human and rat liver cytosols and S105 from <i>Salmonella</i> reduced both dinitropyrenes to II; however, in mammalian microsomal preparations incubated under similar conditions with I, reduced formation of nitrosopyrenes was reported.</p> <p>DNA binding was also affected by oxygen tension: Although binding to calf thymus DNA occurred under aerobic and anaerobic conditions, binding was enhanced under aerobic conditions in incubations containing rabbit lung S9 and I. It was suggested that since some nitroreductases (e.g., NADPH-cytochrome-P-450 reductase [found in microsomal fraction] and xanthine oxidase [found in cytosolic fraction] are known to be inhibited by oxygen, this could have inhibited the production of stable metabolites in this study (Tee et al., 1988) [and those mentioned above]. Apigenin and tannic acid inhibited the bacterial mutagenesis induced by 1,6-DNP by inactivating the nitroreductase activity (Kuo et al., 1992).</p>

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
1,8-Dinitropyrene; 1,8-DNP [42397-65-9] (I)	Unchanged in rat feces (Beland et al., 1988)	Human liver HepG2 cell line (Silvers et al., 1994). Human and rat liver microsomes (Shimada et al., 1990). Parent CHL cells and CHL cells expressing human NAT1 or NAT2 <i>N</i> -acetyltransferase; CHL cell line that stably expressed <i>O</i> -acetyltransferase of <i>S. typhimurium</i> (Watanabe et al., 1994). CHO and V79 cells (O'Donovan, 1990; Srivastava and Wiebel, 1990).	<i>S. typhimurium</i> TA98, YG1021, and YG1026 (Mermelstein et al., 1982; McCoy et al., 1983; Heflich et al., 1985; Watanabe et al., 1989; Einistö et al., 1991). <i>S. typhimurium</i> TA98 and TM677 and rat liver S9 (Mermelstein et al., 1982; Shah et al., 1990, 1991; Busby et al., 1994)	Human, P-450 IIIA4. Rabbit liver, NADPH-cytochrome P-450 reductase. Rabbit, xanthine oxidase, DT-diaphorase, and aldehyde oxidase. Rat, P-450 IA1 and IA2. (Tee et al., 1988; Shimada et al., 1990). Human NAT1 and NAT2 <i>N</i> -acetyltransferase (Watanabe et al., 1994). Acetylation (O'Donovan, 1990; Srivastava and Wiebel, 1990). V79 lung cells acetyltransferase activity of 200 nmol/min/mg protein (Srivastava and Wiebel, 1990).	Nitroreduction was the major metabolic pathway in <i>S. typhimurium</i> (Heflich et al., 1985), and in the several strains studied, increased nitroreductase activity of YG1021 and YG1026 did not result in increased mutagenicity when compared to their parent strains (Watanabe et al., 1989; Einistö et al., 1991). [These data support Fifer et al. (1986a), who stated that nitrated pyrenes are metabolized to mutagens through nitroreduction, and that in <i>Salmonella</i> the limiting step in the activation of I is thought to be subsequent esterification of the reduced intermediates. Djurić et al. (1986b) stated that the reason for I mutagenicity in <i>S. typhimurium</i> is that this species is able to acetylate I. The authors also stated that while <i>N</i> -acetylation may be a detoxification pathway, <i>O</i> -acetylation of <i>N</i> -hydroxylamine derivatives of I enhances DNA binding. McCoy et al (1983), Orr et al. (1985), Fifer et al. (1986b), and Einistö et al. (1991) reported similar findings using the acetyltransferase deficient strain TA98/1,8-DNP ₆ ; Studies using strains YG1024 and YG1029 (both have elevated <i>O</i> -acetyltransferase activity) showed that these strains were more susceptible to I mutagenicity than their parent strains, suggesting the importance of <i>O</i> -acetylation in mutagenicity in <i>S. typhimurium</i> (Watanabe et al., 1990).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
1,8-Dinitropyrene; 1,8-DNP [42397-65-9] (I) (contd.)					Metabolic inference derived from sensitivity to mutagenic effects: NAT2 > NAT1 > parent CHL cells, indicating that both human NAT1 and NAT2 are involved in the metabolic activation of I. Furthermore, the CHL cell line that stably expressed <i>O</i> -acetyltransferase derived from <i>S. typhimurium</i> was more sensitive than parent CHL cell, but less sensitive than cells expressing human <i>O</i> -acetyltransferase (Watanabe et al., 1994) [suggesting that human NAT1 and NAT2 metabolically activate I to a greater extent than <i>S. typhimurium O</i> -acetyltransferase] (see section 5.0 and related table).
Metabolites					
1-Nitroso-6-nitropyrene [101043-65-6]; 1-NO-6-NP (II)		Human liver microsomes and cytosol (Beland, 1986). Rat liver and mammary cytosol (King et al., 1986; Djurić and McGunagle, 1989, Beland et al., 1988).	<i>S. typhimurium</i> (Beland, 1986).	Reduction of II catalyzed by xanthine oxidase (~3 nmol/min/mg) (Djurić and McGunagle, 1989).	Major metabolite detected; further reduction of this metabolite resulted in formation of DNA-reactive species. Metabolite is reoxidized and can result in formation of reactive III (Djurić and McGunagle, 1989). Nitroreductase activity not affected by O ₂ (Djurić and McGunagle, 1989).
1-Nitroso-8-nitropyrene; 1-NO-8-NP [100593-23-5] (II)		Human liver microsomes and cytosol (Djurić et al., 1986b; Heflich et al., 1986; Beland, 1986). Rat liver cytosol and microsomes and mammary cytosol (Djurić et al., 1986b; Heflich et al., 1986; King et al., 1986; Beland, 1986). CHO cell cytosol (Djurić et al., 1986b; Heflich et al., 1986).	<i>S. typhimurium</i> (Djurić et al., 1986b; Heflich et al., 1986; Beland et al., 1988).	Rabbit lung NADPH-cytochrome P-450 reductase, xanthine oxidase, DT-diaphorase, and aldehyde oxidase as postulated by Tee et al. (1988).	

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
1-Nitro-6-nitrosopyrene		CHO cells (Fifer et al., 1986a; see comments).	<i>S. typhimurium</i> (Fifer et al., 1986a; see comments).	Nitroreduction and subsequent acetylation (Fifer et al., 1986a).	Both 1-nitro-6- and -8-nitrosopyrene showed similar levels of mutagenicity in <i>S. typhimurium</i> TA98 and TA98NR (nitroreductase-deficient strain); however, strain TA98/1,8-DNP ₆ (esterificase-deficient strain) showed much lower mutagenic activity. In CHO cells (lacking nitroreductase activity), both nitrosopyrene derivatives were highly mutagenic. These data suggest that nitrated pyrenes are metabolized to mutagens through nitroreduction, and that in <i>Salmonella</i> the limiting step in the activation of I is thought to be subsequent esterification of the reduced intermediates (Fifer et al., 1986a).
1-Nitro-8-nitrosopyrene		CHO cells (Fifer et al., 1986a; see comments).	<i>S. typhimurium</i> (Fifer et al., 1986a; see comments).	Nitroreduction and subsequent acetylation (Fifer et al., 1986a).	See above comments for 1-nitro-6-nitrosopyrene.
<i>N</i> -Hydroxy-1-amino-6-nitropyrene; 1-Hydroxylamino-6-nitropyrene; 1-HONH-6-NP (III)		Rat liver cytosol (Djurić et al., 1985; Djurić and McGunagle, 1989)		Cytosolic nitroreductases; nitroreduced (Tokiwa and Ohnishi, 1985; Beland, 1986).	Formed under aerobic and anaerobic conditions. Cannot be quantified directly, but reacts readily with DNA following AcCoA <i>O</i> -acetylation (Djurić and McGunagle, 1989).
<i>N</i> -Hydroxy-1-amino-8-nitropyrene; 1-Hydroxylamino-8-nitropyrene; 1-HONH-8-NP (III)		Rat liver cytosol (Djurić et al., 1985).		Cytosolic nitroreductases (Tokiwa and Ohnishi, 1985).	I binding to DNA not detected when using dog liver cytosol, which is known to be deficient in acetylase (Djurić et al., 1985).
Arylhydroxamic acid: <i>N</i> -Hydroxy- <i>N</i> -acetyl-1-amino-1,6- (or -1,8-)dinitropyrene (IV)				AcCoA (Beland, 1986).	Postulated intermediate (Beland, 1986).
<i>N</i> -Acetoxy-1-amino-6-nitropyrene (V)		Rat liver cytosol (Djurić et al., 1985).		<i>N,O</i> -Acetyltransferase and/or AcCoA-dependent oxidases (Tokiwa and Ohnishi, 1985; Beland, 1986).	Two postulated pathways: IV is converted into V by <i>N,O</i> -acetyltransferase and/or V may be formed by direct <i>O</i> -acetylation (Beland, 1986).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
N-Acetoxy-1-amino-8-nitropyrene (V)		Rat liver cytosol (Djurić et al., 1985).		N,O-acetyltransferase and/or AcCoA (Beland, 1986).	Two postulated pathways: IV is converted into V by N,O-acetyltransferase and/or V may be formed by direct O-acetylation. The presence of acetylated metabolites and higher II binding observed in conventional rats suggests that hepatic O-acetylation is important for the metabolic activation of I and II <i>in vivo</i> (Beland, 1986).
Pyrenylnitrenium ion (VI)					Postulated by Beland (1986) as the immediate precursor to IX.
1-Amino-6-nitropyrene; [30269-01-3] (VII)		Rat liver microsomes and cytosol and mammary cytosol and hepatocytes (Djurić et al., 1985; King et al., 1986; Beland, 1986; Drummond et al., 1992 abstract). CHO cells (Beland et al., 1988).	<i>S. typhimurium</i> (Beland, 1986).	See Comments (Djurić et al., 1985).	The addition of AcCoA to the incubations increased the binding of DNPs 20- to 40-fold (Djurić et al., 1985).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
1-Amino-8-nitropyrene; [30269-02-4] (VII)	Rat feces (Heflich et al., 1986; Beland et al., 1988).	Human liver cytosol (Djurić et al., 1986b; Heflich et al., 1986). Human, Rhesus monkey, and rat intestinal contents (Cerniglia et al., 1986 abstract; cited by IARC, 1989). Rat liver cytosol and microsomes, mammary gland cells, and mammary cytosol (Djurić et al., 1985 and 1986; Heflich et al., 1986; King et al., 1986; Imaida et al., 1988; Beland, 1986). CHO cell cytosol (Djurić et al., 1986b; Heflich et al., 1986; Beland et al., 1988).	<i>S. typhimurium</i> (Quillium et al., 1982; Bryant et al., 1984; Tokiwa et al., 1985; Djurić et al., 1986b; Heflich et al., 1985 and 1986; Beland, 1986). <i>Clostridium leptum</i> , <i>C. paraputrificum</i> , <i>C. clostridiiforme</i> , and a <i>Eubacterium</i> species (Rafii et al., 1991).		Enzymatic activity other than (or in addition to) nitroreductase is required for the activation of 1,8-DNP (Bryant et al., 1984). Major metabolite formed, with further activation occurring <i>via</i> acetylation subsequent to nitroreduction (Heflich et al., 1985; in agreement with Bryant et al., 1984, and Quillium et al., 1982).
1,6-Diaminopyrene; 1,6-DAP		Rat mammary cytosol (King et al., 1986).	<i>S. typhimurium</i> (Beland, 1986).		
1,8-Diaminopyrene; 1,8-DAP [30269-04-6]	Rat feces (Heflich et al., 1986; Beland et al., 1988).	Rhesus monkey intestinal contents (Cerniglia et al., 1989). Rat mammary cytosol (King et al., 1986).	<i>S. typhimurium</i> (Bryant et al. 1984; Orr et al., 1985; Tokiwa et al., 1985; Beland, 1986).	Subsequently acetylated by AcCoA (Tokiwa et al., 1985).	Subsequent acetylated to VIII and 1,8- <i>N,N'</i> -diacetylamino-pyrene in <i>S. typhimurium</i> TA98NR (Orr et al., 1985).
1-Acetylamino-6-nitropyrene; 1-Acetamido-6-aminopyrene [99387-36-7] VIII		Chinese hamster embryo (CHE) cells (Neft et al., 1993).	<i>S. typhimurium</i> (Beland, 1986).		1,6-DNP was nitroreduced and subsequently acetylated in CHE cells (Neft et al., 1993).
1-Acetylamino-8-nitropyrene; 1-Acetamido-8-aminopyrene [99387-36-7] VIII	Rat feces (Heflich et al., 1986; Beland et al., 1988).	Rat mammary gland cells (Imaida et al., 1988). Rabbit lung (Tee et al., 1988).	<i>S. typhimurium</i> (Beland, 1986; Orr et al., 1985).	AcCoA dependent (Tee et al., 1988).	Higher binding to calf thymus DNA in the presence of O ₂ (Tee et al., 1988).

Table 6-1. 1,6- and 1,8-Dinitropyrene Metabolite and Adduct Identification (Continued)

Name [CASRN] No. ^a	<i>In Vivo</i>	<i>In Vitro</i>	Bacteria	Reaction/Enzymes	Comments
<i>N,N'</i> -Diacetyl-1,6-diaminopyrene; 1,6-Diacetamidopyrene			<i>S. typhimurium</i> (Beland, 1986)	From 1,6-diaminopyrene	
<i>N,N'</i> -Diacetyl-1,8-diaminopyrene; 1,8-Diacetamidopyrene	Rat feces (Heflich et al., 1986; Beland et al., 1988).		<i>S. typhimurium</i> (Orr et al., 1985; Beland, 1986).	From 1,8-diaminopyrene and catalyzed by AcCoA (Orr et al., 1985).	Acetylation is an important process in the metabolic activation of 1,8-DNP to the mutagenic intermediate in <i>S. typhimurium</i> (Orr et al., 1985).
Adducts					
<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-6-NP (IX)	Rat liver, mammary glands, peripheral blood lymphocytes, kidney, and urinary bladder (Djurić et al., 1988; El-Bayoumy et al., 1994). Rat (male) lung (target tissue), liver, WBC, and spleen lymphocytes (surrogate tissues) (Smith et al., 1995).	Rat liver cytosols in the presence of calf liver DNA (Djurić et al., 1988)	<i>S. typhimurium</i> (Djurić et al., 1986b; Heflich et al., 1985a; Andrews et al., 1986; Beland, 1986).	AcCoA involved in pathway (Imaida et al., 1988; Djurić et al., 1985, 1988). Nitrenium ion intermediates (Beland, 1986).	DNA adduct postulated to form via a substitution at C8 of dG with nitrenium ion intermediates (Beland, 1986). Radioactivity bound to the heme moiety of hemoglobin (El-Bayoumy et al., 1994). Incubations including rat mammary cytosol and I (1,6- or 1,8-) in the presence of AcCoA formed metabolite binding to tRNA (Imaida et al., 1988).
<i>N</i> -(Deoxyguanosin-8-yl)-1-amino-8-NP (IX)	Rat livers and mammary glands (Beland, 1986).		<i>S. typhimurium</i> (Djurić et al., 1986b; Heflich et al., 1985a; Andrews et al., 1986; Beland, 1986).	AcCoA involved in activation pathway (Djurić et al., 1985).	The initial reduction of I and II to <i>N</i> -hydroxyamino-mononitro derivatives is an activation pathway (Fu, 1990). DNA adduct postulated to form via a substitution at C8 of dG with nitrenium ion intermediates (Beland, 1986). No apparent correlation between AcCoA and activation of I (1,8-DNP) to a reactive DNA adduct (Tee et al., 1988).

^a A number corresponds to Roman numeral used for metabolite identification in Figure 6-1.

Table 6-2. Cell Proliferation Induced by 1,6- and 1,8-Dinitropyrene

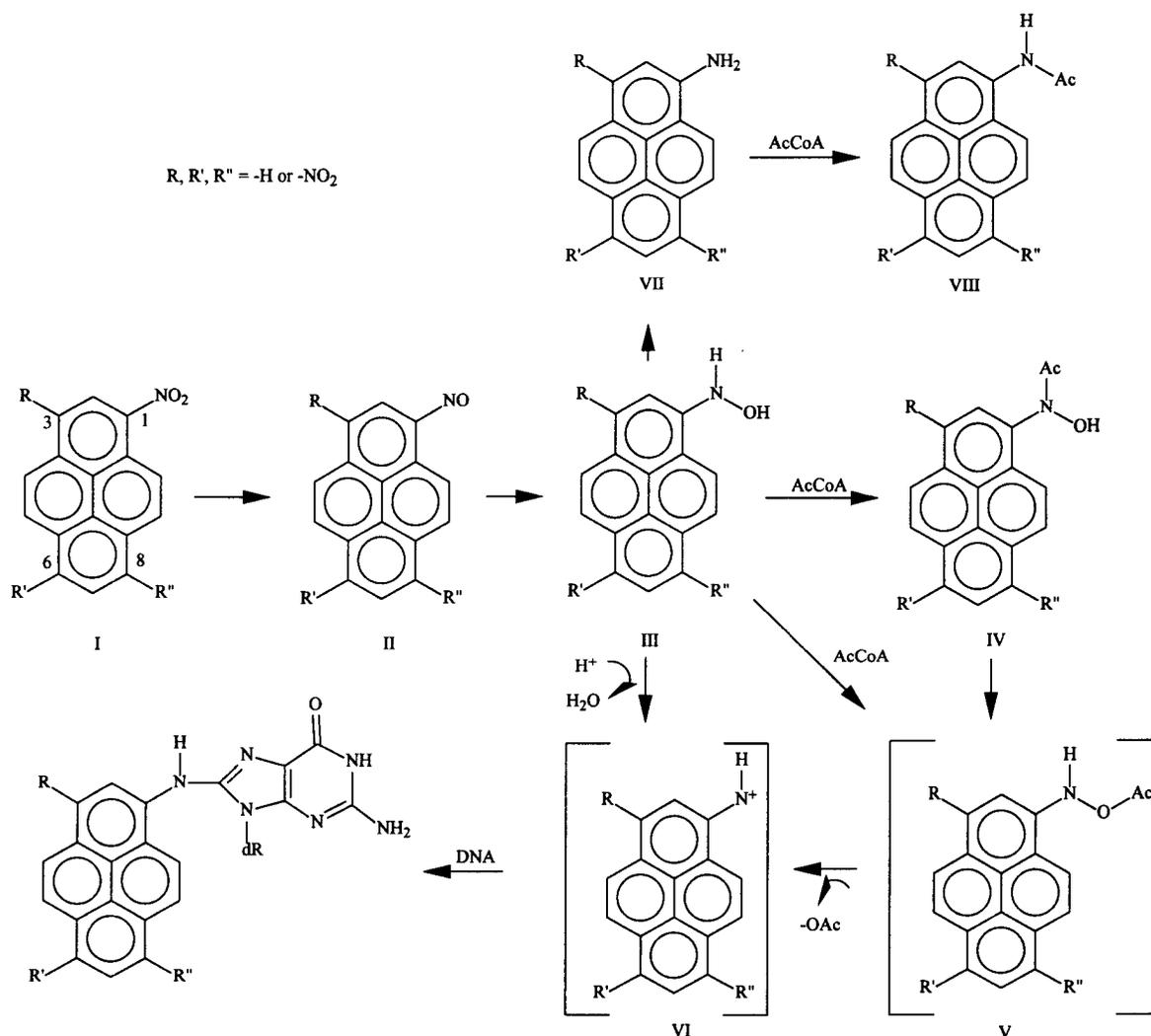
Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - Oral Administration							
weanling CD	36F	36F (DMSO alone)	1,6- and 1,8-DNP, >99% pure	10 µmol/kg bw in DMSO (1.7 µmol/mL DMSO), 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>Statistical analyses were not performed to determine the significance of chemically induced hyperplasia.</p> <p>Kidney: Positive (for proliferative activity as indicated by presence of hyperplasia)</p> <p>The incidence of pelvic hyperplasia was increased in 1,6- and 1,8-DNP-treated rats (1,6-DNP: 12/36 vs. 5/36 controls; 1,8-DNP: 12/36 vs. 5/36 controls).</p> <p>Liver: Positive (for proliferative activity as indicated by presence of altered foci and hyperplasia; 1,8-DNP only)</p> <p>The incidences of cellular altered foci and hyperplastic nodules were increased in 1,8-DNP-treated, but not 1,6-DNP-treated, rats (altered foci: 5/36 vs. 2/36 controls; hyperplastic nodules: 6/36 vs. 2/36 controls).</p> <p>Other: Negative</p> <p>There was no increase in proliferative activity in other examined tissues of DNP-treated rats.</p>	King (1988); Imaida et al. (1991b)

Table 6-2. Cell Proliferation Induced by 1,6- and 1,8-Dinitropyrene (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	effective number: 23F	effective number: 31F (DMSO alone)	1,6- and 1,8-DNP, >99% pure	10 µmol/kg bw in DMSO (1.7 µmol/mL DMSO), 3 times/wk	4 wk	<p>Rats were killed when moribund or after 76-78 weeks. Adrenal glands, pituitary gland, liver, pancreas, kidneys, thyroid gland, blood, and mammary glands were examined histologically.</p> <p>Statistical analyses were not performed to determine the significance of chemically induced hyperplasia.</p> <p>All Tissues: Negative</p> <p>There was no increase in proliferative activity in examined tissues of DNP-treated rats.</p>	King (1988); Imaida et al. (1991b)

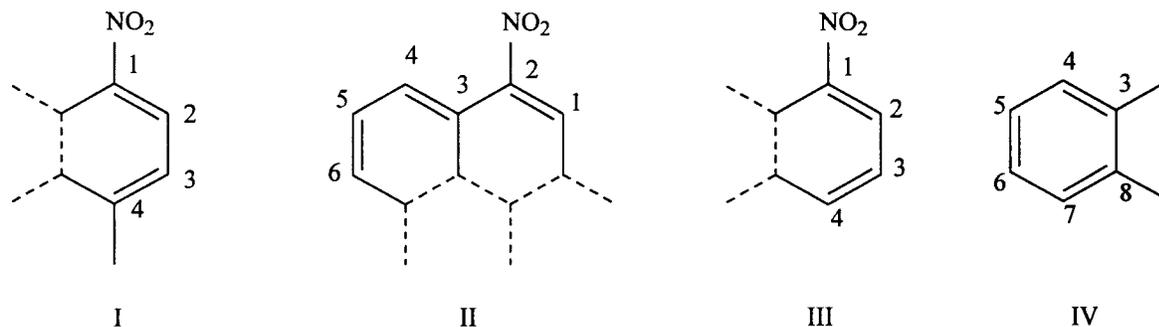
Abbreviations: bw = body weight; DMSO = dimethyl sulfoxide; F = females; M = males

Figure 6-1. Proposed Mechanism for Activation of Nitrated Pyrenes to DNA-Binding Species



Proposed mechanism for activation of nitrated pyrenes (I) to DNA-binding species. The compounds undergo sequential reduction to nitrosopyrenes (II) and *N*-hydroxy aminopyrenes (III). With 1-nitropyrene, the *N*-hydroxy amino metabolite reacts directly with DNA, while the analogous metabolites from the dinitropyrenes are further activated by AcCoA. This AcCoA-dependent binding may occur through the formation of an aryhydroxamic acid (IV) which can be converted into a reactive *N*-acetoxy arylamine (V) by *N,O*-acyltransferase. Alternatively, the *N*-acetoxy arylamine may be formed by direct *O*-acetylation of the *N*-hydroxy aminopyrene. In all cases, the predominant adducts formed are substituted at C-8 of deoxyguanosine and arise through nitrenium ion intermediates (VI). *N*-Hydroxy aminopyrenes can also be further reduced to aminopyrenes (VII) and subsequently acetylated to *N*-acetylaminopyrenes (VIII) (Beland, 1986).

Figure 6-2. 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 CASRN's 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 Code	Definition
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, postspematogonia
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