2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 2-nitrophenol and 4-nitrophenol and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for 2-nitrophenol and 4-nitrophenol based on toxicological studies and epidemiological investigations.

Mononitrophenols exist in three isomeric forms: 2-nitrophenol (or o-nitrophenol), 3-nitrophenol (or m-nitrophenol), and 4-nitrophenol (or p-nitrophenol). Because of a scarcity of toxicological data regarding 3-nitrophenol and because this isomer is much less prevalent in industry and in the environment, only 2-nitrophenol and 4-nitrophenol are discussed in this document.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELS) reflect *the* actual doses (levels of exposure) used in *the* studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should also help to determine whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates

of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability from laboratory animal data to humans.

Although methods have been established to derive these levels (Barnes et al. 1988; EPA 1989), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effect data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

2.2.1 Inhalation Exposure

Two studies were identified that examined the effects of inhalation exposure to nitrophenols (Hazleton 1983; Smith et al. 1988). These studies described the effects of acute- and intermediate-duration exposure to 4-nitrophenol in rats. The results are presented in relevant sections below.

2.2.1.1 Death

No studies were located regarding lethality in humans or animals following inhalation exposure to 2-nitrophenol or in humans following inhalation exposure to 4-nitrophenol.

No lethality was observed in male rats exposed to dust atmospheres of 4-nitrophenol (sodium salt) at concentrations of 4,033 mg 4-nitrophenol/m^3 for a single 4-hour period (Smith et al. 1988), to 2,119 mg 4-nitrophenol/m^3 for 6 hours/day for 10 days (Smith et al, 1988), or in rats (both sexes) exposed to 30 mg 4-nitrophenol dust/m^3 for 6 hours/day, 5 days/week for 4 weeks (Hazleton 1983). The NOAELs are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

No studies were located regarding systemic effects in humans or animals following inhalation exposure to 2-nitrophenol or in humans following inhalation exposure to 4-nitrophenol.

Data regarding systemic effects of 4-nitrophenol following inhalation exposure were limited to two studies. These studies examined the effects of acute- and intermediate-duration exposure of rats to 4-nitrophenol for the following systemic categories: respiratory, cardiovascular, gastrointestinal,

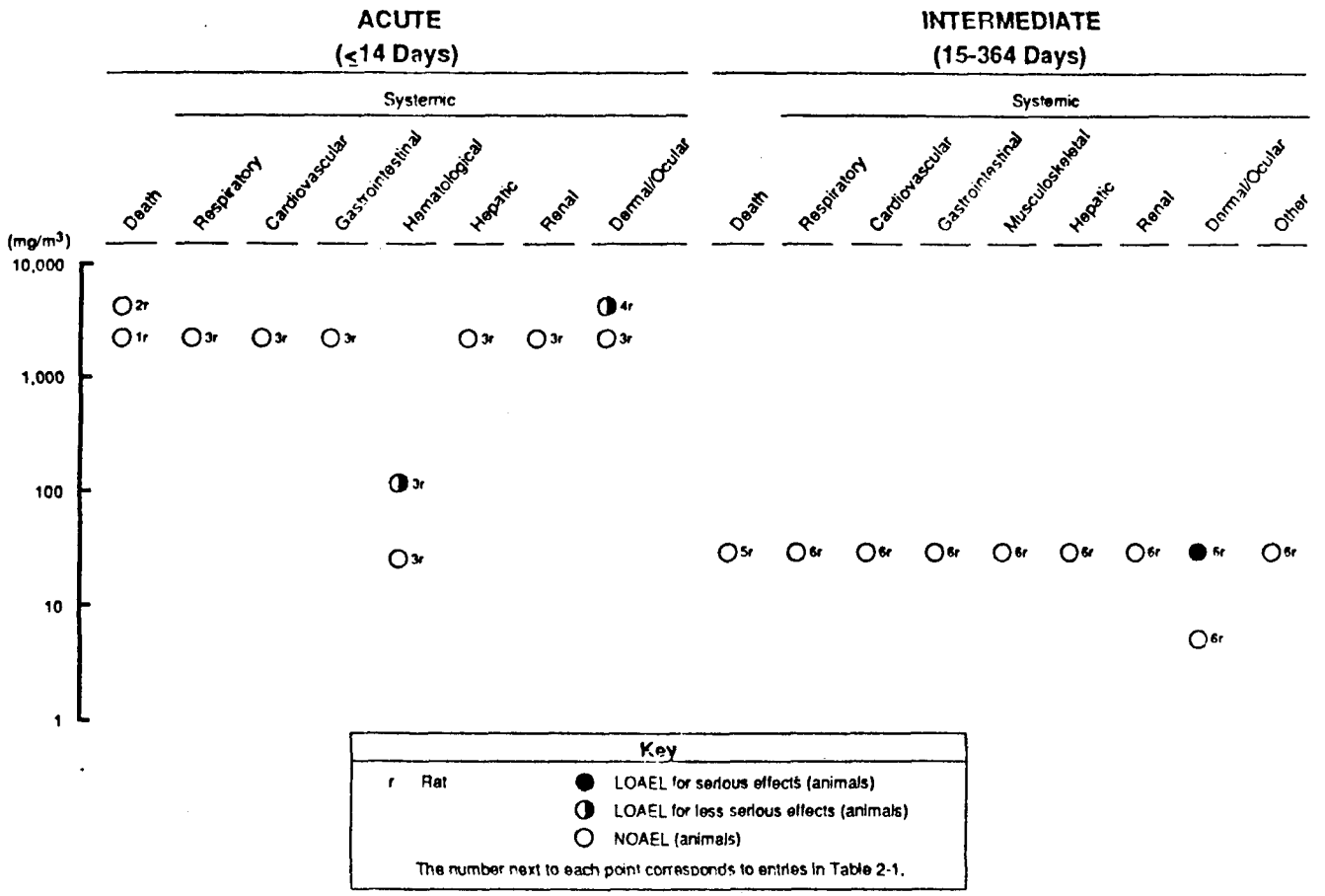
TABLE 2-1. Levels of Significant Exposure to 4-Nitrophenol - Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (mg/m^3)	LOAEL (effect) Less serious (mg/m^3)	LOAEL (effect) Serious (mg/m^3)	Reference
ACUTE EXPOSURE							
Death							
1	Rat	2 wk 5 d/wk 6 hr/d		2,119			Smith et al. 1988
2	Rat	1 d 4 hr/d		4,033			Smith et al. 1988
Systemic							
3	Rat	2 wk 5 d/wk 6 hr/d	Resp Cardio Gastro Hemato Hepatic Renal Derm/oc	2,119 2,119 2,119 26 2,119 2,119 2,119	112 (methemoglobinemia)		Smith et al. 1988
4	Rat	1 d 4 hr/d	Derm/oc		4,033 (corneal opacity)		Smith et al. 1988
INTERMEDIATE EXPOSURE							
Death							
5	Rat	4 wk 5 d/wk 6 hr/d		30			Hazleton 1983
Systemic							
6	Rat	4 wk 5 d/wk 6 hr/d	Resp Cardio Gastro Musc/sk Hepatic Renal Derm/oc Other	30 30 30 30 30 30 5 30		30 (anterior capsular cataract 11/30)	Hazleton 1983

[a]The number corresponds to entries in Figure 2-1.

d = day; Cardio = cardiovascular; Derm/oc = dermal/ocular; Gastro = gastrointestinal; Hemato = hematological; hr = hours; LOAEL = lowest-observed-adverse-effect level; mg/m^3 = milligram per cubic meter; Musc/sk = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = weeks

FIGURE 2-1. Levels of Significant Exposure to 4-Nitrophenol - Inhalation



hematological, musculoskeletal, hepatic, renal, dermal/ocular, and other systemic. The highest NOAEL values and all reliable LOAEL values for each systemic effect are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. Rats exposed to dust atmospheres of 4-nitrophenol (sodium salt) at a concentration of 2,119 mg 4-nitrophenol/m^3, 6 hours/day for 10 days showed a decrease in absolute and relative lung weights after a 14-day recovery period (Smith et al. 1988). Since no histopathological changes were noticed, the biological significance of this finding is unclear. A concentration of 292 mg/m^3 was without effect. The concentration of 2,119 mg/m^3, is considered a NOAEL for respiratory effects for acute-duration exposure. Male and female rats exposed to 30 mg 4-nitrophenol dust/m^3 6 hours/day, 5 days/week for 4 weeks showed no exposure-related effects on lung weight, or on gross and histological appearance of the lungs, trachea, and nasal turbinates (Hazleton 1983). This exposure level represents a NOAEL for respiratory effects for intermediate-duration exposure.

Cardiovascular Effects. No exposure-related histopathological lesions or increased weights were observed in the hearts of male rats exposed for 2 weeks to up to 2,119 mg 4-nitrophenol/m^3 as dusts of the sodium salt (Smith et al. 1988). Similarly, no cardiac effects were observed in male and female rats exposed intermittently to up to 30 mg of 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). These two exposure levels are considered NOAELs for cardiovascular effects for acute- and intermediate-duration exposure, respectively, although no further tests for cardiovascular function were performed.

Gastrointestinal Effects. Male rats exposed for 2 weeks to up to 2,119 mg 4-nitrophenol/m^3 as dusts of the sodium salt had no histopathological alterations in the esophagus, stomach, small intestine, colon, and cecum (Smith et al. 1988). Similar results were reported in male and female rats exposed to up to 30 mg of 4-nitrophenol dusts/m^3 for 4 weeks (Hazleton 1983).

Hematological Effects. Rats exposed to 112 mg of 4-nitrophenol/m^3 as 4-nitrophenol sodium salt for 2 weeks showed a significant ($p<0.05$) increase in methemoglobin, but exposure to 26 mg/m^3 was without effect (Smith et al. 1988). After a 14-day recovery period, methemoglobin levels were reduced but had not reached preexposure values. In a similar experimental series, which used exposure concentrations of 292 and 2,119 mg 4-nitrophenol/m^3, the increase in methemoglobin was dose-related. Rats exposed to up to 30 mg 4-nitrophenol dusts/m^3 6 hours/day, 5 days/week for 4 weeks showed no significant alterations in hematology parameters (Hazleton 1983). Methemoglobin values, however, determined after 2 weeks of exposure, showed great variability, and appeared to be unusually high (greater than 3%) for some unexposed animals (normal is about 0.5%).

Musculoskeletal Effects. Rats exposed to up to 30 mg 4-nitrophenol dusts/m^3 for 6 hours/day, 5 days/week for 4 weeks showed no exposure-related effects on the gross or microscopical appearance of the femur and skeletal muscles (Hazleton 1983).

Hepatic Effects. Slightly increased levels of serum glutamic oxaloacetic transaminase (SGOT) were found in rats exposed for 2 weeks to a dust of 4-nitrophenol sodium salt at concentrations of 292 and 2,119 mg 4-nitrophenol/m^3 (Smith et al. 1988). However, the toxicological significance of the increase is unclear. In addition, no histological evidence of liver damage was found. No exposure-related effects on liver weight or on the gross and histological appearance of the liver was observed in rats exposed to up to 30 mg 4-nitrophenol dusts/m^3, 6 hours/day for 4 weeks (Hazleton 1983). In addition, this exposure protocol did not alter serum levels of SGOT or serum glutamic pyruvic transaminase (SGPT).

Renal Effects. Rats exposed to 292 or 2,119 mg 4-nitrophenol/m^3 of 4-nitrophenol dust (sodium salt) for 2 weeks had darker urine and proteinuria (Smith et al. 1988). In the absence of further information, and because no histopathological changes were noticed in the kidneys, the significance of this finding is unclear. Rats exposed to up 30 mg 4-nitrophenol dust/m^3 for 4 weeks showed no exposure-related effects on kidney weight, or on the gross and microscopical appearance of the kidneys (Hazleton 1983).

Dermal/Ocular Effects. Cornea1 opacity was described in 4 of 6 rats exposed to a concentration of 4,033 mg 4-nitrophenol/m^3 as 4-nitrophenol dust (sodium salt) for 4 hours (Smith et al. 1988) (see Table 2-1 and Figure 2-l). The effect persisted through a 14-day observation period in one rat. This effect may be due to direct contact of 4-nitrophenol with the cornea and, as such, could also be classified under effects caused by dermal exposure. Exposure to a concentration of 2,119 mg 4-nitrophenol/m^3 6 hours/day for 2 weeks was without effect. Unilateral and bilateral diffuse anterior capsular cataracts were observed in male and female rats exposed to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). This exposure level is presented as a LOAEL for intermediate duration exposure in Table 2-1. An exposure level of 5 mg/m^3 was without effect.

Other Systemic Effects. No histological alterations were reported in the spleens and thyroid glands of male rats exposed for 2 weeks to up to 2,119 mg 4-nitrophenol/m^3 as 4-nitrophenol dust (Smith et al. 1988), but no additional information was provided. No consistent exposure-related effects on body weight were reported in rats exposed to up to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). In addition, no gross or histological alterations were observed in the urinary bladder, thyroid and parathyroid glands, pituitary, salivary glands, adrenals, pancreas, and mammary glands (Hazleton 1983).

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals following inhalation exposure to 2-nitrophenol or in humans following inhalation exposure to 4-nitrophenol.

No histological alterations were observed in lymph nodes, thymus, and sternal bone marrow of rats exposed for 2 weeks to up to 2,119 mg 4-nitrophenol/m^3 as dust of the sodium salt (Smith et al. 1988). Similar results were reported in rats exposed to up to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). However, since no immunological tests were performed in these studies, reliable NOAELs for immunological effects cannot be determined.

2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals following inhalation exposure to 2-nitrophenol or in humans following inhalation exposure to 4-nitrophenol.

No histological alterations were observed in the brains of rats exposed for 2 weeks to a dust of 4-nitrophenol sodium salt at concentrations of up to 2,119 mg 4-nitrophenol/m^3 (Smith et al. 1988). Gross and histological examination of the brain, spinal cord, and peripheral nerves of rats exposed to up to 30 mg 4-nitrophenol dust/m^3 for 4 weeks revealed no treatment-related effects (Hazleton 1983). However, since neurological tests were not performed in these studies, reliable NOAELs for neurological effects cannot be determined.

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals following inhalation exposure to 2-nitrophenol or 4-nitrophenol.

2.2.1.6 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals following inhalation exposure to 2-nitrophenol or in humans following inhalation exposure to 4-nitrophenol.

Male rats exposed for 2 weeks to a dust of 4-nitrophenol sodium salt at concentrations of up to 2,119 mg 4-nitrophenol/m^3 showed no histological alterations in the testes and epididymides (Smith et al. 1988). Rats exposed to up to 30 mg 4-nitrophenol dust/m^3 for 4 weeks showed no exposure-related effects on the gross or microscopical appearance of the prostate, seminal vesicles, ovaries, or uterus (Hazleton 1983). Nevertheless, since tests for reproductive performance were not conducted in these studies, reliable NOAELs for reproductive effects cannot be determined.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or Animals following inhalation exposure to 2-nitrophenol or 4-nitrophenol.

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding the carcinogenic effects in humans or animals following inhalation exposure to 2-nitrophenol or 4-nitrophenol.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding lethality in humans following oral exposure to 2-nitrophenol or 4-nitrophenol.

In rats, the reported oral LD_{5O} values after gavage administration of 2-nitrophenol and 4-nitrophenol in corn oil were 2,830 and 620 mg/kg, respectively (Vernot et al. 1977). An LD_{5O} value of 230 mg/kg was reported in albino rats for 4-nitrophenol administered in propylene glycol (Monsanto 1983a); clinical observations prior to death included convulsions, prostration, and dyspnea. Twenty-three percent lethality was reported in pregnant rats administered a single dose of 667 mg 4-nitrophenol/kg on day 11 of gestation; a dose of 333 mg/kg was without effect (Kavlock 1990). Early mortality was reported in rats administered 70 mg 4-nitrophenol/kg or more by gavage in water for 13 weeks (Hazleton 1989); prostration, wheezing, and dyspnea were noticed prior to death. In mice, LD_{5O} values of 470 mg/kg (Vernot et al. 1977) and 626 mg/kg (Plasterer et al. 1985) have been reported for 4-nitrophenol and 1300 mg/kg for 2-nitrophenol (Vernot et al. 1977) after gavage administration of the chemicals in corn oil. In addition to determining an LD_{5O} in mice, Plasterer et al. (1985) reported that daily gavage doses of 400 mg of 4-nitrophenol/kg administered to pregnant mice during gestation days 7-15 caused 19% lethality. Three deaths were reported in eight female rabbits given 4-nitrophenol in single gavage doses between 182 and 322 mg/kg (Williams 1938); the lowest lethal dose was 220 mg 4-nitrophenol/kg. The cause of death was not indicated in any of these studies. Although the data regarding lethality are limited, 4-nitrophenol is apparently more lethal than 2-nitrophenol. The LD_{5O} values and other doses causing death are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans or animals following oral exposure to 2-nitrophenol. Data regarding systemic effects of

TABLE 2-2. Levels of Significant Exposure to Nitrophenols - Oral

Key to figure[a]	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Isomer
ACUTE EXPOSURE									
Death									
1	Rat	(GO)	NS				2830 (LD_{50})	Vernot et al. 1977	2-
2	Rat	(GW)	1x Gd 11		333		667 (3/13 deaths)	Kavlock 1990	4-
3	Rat	(GW)	1x		110		230 (LD_{50})	Monsanto 1983a	4-
4	Rat	(GO)	NS				620 (LD_{50})	Vernot et al. 1977	4-
5	Rabbit	(GW)	1x				220 (3/8)	Williams 1938	4-
6	Mouse	(GO)	8 d 1x/d				626 (LD_{50})	Plasterer et al. 1985	4-
7	Mouse	(GO)	NS				1300 (LD_{50})	Vernot et al. 1977	2-
8	Mouse	(GO)	8 d 1x/d				400 (19%)	Plasterer et al. 1985	4-
9	Mouse	(GO)	NS				470 (LD_{50})	Vernot et al. 1977	4-
Developmental									
10	Rat	(GW)	1x Gd 11		1000			Kavlock 1990	4-
INTERMEDIATE EXPOSURE									
Death									
11	Rat	(GW)	13 wk 7 d/wk		25		70 (3/13)	Hazleton 1989	4-

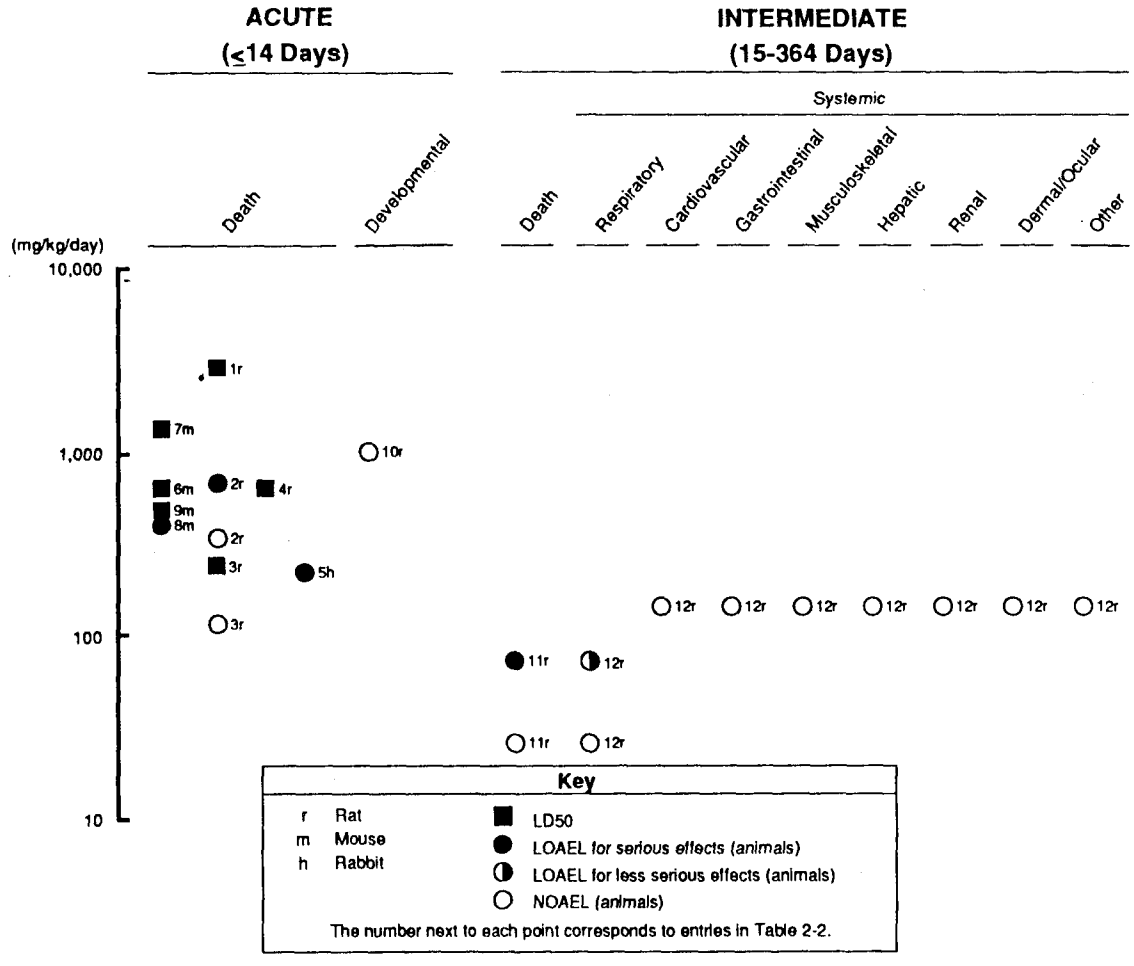
TABLE 2-2 (Continued)

Key to figure[a]	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Isomer
12	Rat	(GW)	13 wk 7 d/wk	Resp	25	70 (wheezing, dyspnea)		Hazleton 1989	4-
				Cardio	140				
				Gastro	140				
				Musc/sk	140				
				Hepatic	140				
				Renal	140				
				Derm/oc	140				
				Other	140				

[a]The number corresponds to entries in Figure 2-2.

Cardio = cardiovascular; d = day; Derm/oc = dermal/ocular; Gastro = gastrointestinal; Gd = gestation day; GO = gavage, oil; GW = gavage, water; LD_{50} = lethal dose 50% kill; LOAEL = lowest-observed-adverse-effect level; Musc/sk = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week; x = times

FIGURE 2-2. Levels of Significant Exposure to 2- and 4-Nitrophenol - Oral



4-nitrophenol following oral exposure were limited to a single study (Hazleton 1989). This study examined the effects of intermediate-duration exposure in rats in the following systemic categories: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal/ocular, and other systemic. The highest NOAEL values and all reliable LOAEL values for each systemic effect are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. No histological alterations were observed in the trachea and lungs of rats administered daily doses of up to 140 mg 4-nitrophenol/kg by gavage for 13 weeks (Hazleton 1989). However, wheezing and dyspnea were observed in rats given doses of 70 mg/kg or more that died prematurely during the study. A dose of 25 mg 4-nitrophenol/kg was without effect.

Cardiovascular Effects. No gross or histological alterations were reported in the heart and aorta of rats administered up to 140 mg 4-nitrophenol/kg/day by gavage in water for 13 weeks (Hazleton 1989).

Gastrointestinal Effects. No treatment-related effects were observed on the gross or microscopical appearance of the esophagus, stomach, duodenum, jejunum, ileum, colon, cecum, and rectum of rats administered up to 140 mg 4-nitrophenol/kg/day by gavage in water for 13 weeks (Hazleton 1989).

Hematological Effects. No significant alterations were observed in hematological and clinical chemistry parameters of rats administered up to 140 mg 4-nitrophenol/kg/day for 13 weeks (Hazleton 1989). Methemoglobin values of untreated rats determined at week 7 were unacceptably high, which led the investigators to suggest that the analytical method was not totally reliable; therefore, methemoglobin was not measured at sacrifice. Since methemoglobin formation appears to be the end point with the lowest threshold in rats following inhalation exposure to 4-nitrophenol (Smith et al. 1988), a reliable NOAEL for hematological effects due to oral exposure cannot be determined based on the findings reported by Hazleton (1989).

Musculoskeletal Effects. Rats administered up to 140 mg 4-nitrophenol/kg/day by gavage in water for 13 weeks showed no gross or histological alterations in the sternum (Hazleton 1989). In addition, no gross alterations were observed in the cranial cavity.

Hepatic Effects. Dark, enlarged, and thicker liver lobes were observed in some rats that died prematurely in a 13-week gavage study with 4-nitrophenol (Hazleton 1989). Early deaths occurred with doses of 70 mg 4-nitrophenol/kg/day or more. However, no gross or histological alterations were observed at sacrifice (13 weeks) in rats that received doses of up to 140 mg 4-nitrophenol/kg/day. Furthermore, serum levels of liver enzymes and bilirubin were unaffected by treatment with 4-nitrophenol.

Renal Effects. Some rats that died early in a 13-week gavage study with 4-nitrophenol had kidney congestion (Hazleton 1989). Early deaths were observed at doses of 70 mg 4-nitrophenol/kg/day or more. Rats administered up to 140 mg 4-nitrophenol/kg/day, and sacrificed at week 13, however, showed no treatment-related effects on gross or histological appearance of the kidneys.

Dermal/Ocular Effects. No treatment-related ophthalmological alterations were reported throughout the experimental period in rats administered up to 140 mg 4-nitrophenol/kg/day by gavage for 13 weeks (Hazleton 1989).

Other Systemic Effects. Rats administered up to 140 mg 4-nitrophenol/kg/day by gavage for 13 weeks showed no significant effects on body weight gain, or on the gross or microscopical appearance of the salivary glands, pituitary, thyroid and parathyroid glands, adrenals, pancreas, and urinary bladder (Hazleton 1989).

2.2.2.3 Immunological Effects

No exposure-related effects were reported on spleen weight, or on the microscopical appearance of spleen, thymus, and lymph nodes of rats administered up to 140 mg 4-nitrophenol/kg/day by gavage for 13 weeks (Hazleton 1989). However, since no immunological tests were performed, a reliable NOAEL for immunological effects cannot be determined.

2.2.2.4 Neurological Effects

No exposure-related effects were reported on brain weight, or on the histological appearance of the brain and sciatic nerve of rats given up to 140 mg 4-nitrophenol/kg/day by gavage for 13 weeks (Hazleton 1989). However, since neurological tests were not performed, a reliable NOAEL for neurological effects cannot be determined.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals following oral exposure to 2-nitrophenol or in humans following oral exposure to G-nitrophenol.

No significant effects on litter size, perinatal loss, pup weight, and litter biomass were observed in rats treated with a single gavage dose of up to 1,000 mg/kg of G-nitrophenol on day 11 of gestation (Kavlock 1990). In addition, no overt malformations were observed, but the pups were not examined for internal malformations. No changes were observed in the reproductive index of pregnant mice given daily doses of 400 mg 4-nitrophenol/kg by gavage during gestation days 7-14 (Plasterer et al. 1985). The 400 mg/kg dose, however, caused 19% maternal lethality. The reproductive index was defined as

the ratio between survivors that delivered and survivors pregnant and is a measure of prenatal lethality. Furthermore, G-nitrophenol did not affect the number of live pups or the average weight of the pups, and produced no gross anomalies. However, the pups were not examined for internal malformations. The NOAEL value of 1,000 mg/kg for developmental effects is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Reproductive Effects

No treatment-related effects were observed on testes weight, or on the histological appearance of the testes, ovaries, and uterus of rats administered up to 140 mg 4-nitrophenol/kg/day by gavage for 13 weeks (Hazleton 1989). However, since tests for reproductive performance were not conducted, a reliable NOAEL for reproductive effects cannot be determined.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following oral exposure to 2-nitrophenol or 4-nitrophenol.

Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer effects in humans or animals following oral exposure to 2-nitrophenol or 4-nitrophenol.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding lethality in humans following dermal exposure to 2-nitrophenol or 4-nitrophenol.

No lethality was reported among rabbits when a saline suspension of 5,000 mg 4-nitrophenol/kg was applied to the abraded dorsal surface for 24 hours (Monsanto 1983b). The animals were observed for 15 days. No treatment-related deaths were observed in rats treated dermally with doses between 50 and 250 mg/kg/day of 4-nitrophenol for 120 days (Angerhofer 1985). In mice, application of a 47 mg/kg/day dose of 2-nitrophenol or 4-nitrophenol to shaved skin for 12 weeks did not alter the survival rate (Boutwell and Bosch 1959). The NOAELs are recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculo/skeletal, hepatic, renal, dermal/ocular, or other systemic effects in humans or animals after dermal exposure to 2-nitrophenol or in humans after dermal exposure to 4-nitrophenol.

TABLE 2-3. Levels of Significant Exposure to Nitrophenols - Dermal

Species	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Isomer
ACUTE EXPOSURE							
Death							
Rabbit	24 hr		5,000			Monsanto 1983b	4-
Systemic							
Rabbit	24 hr	Derm/oc		181 (skin scabbing and scarring)		Monsanto 1983d	4-
Rabbit	4 hr	Derm/oc		147 (skin erythema and edema)		Monsanto 1984	4-
Rabbit	1x	Derm/oc			27 (corneal cloudiness)	Monsanto 1983c	4-
Rabbit	24 hr	Derm/oc		5,000 (erythema and edema)		Monsanto 1983b	4-
INTERMEDIATE EXPOSURE							
Rat	120 d		250			Angerhofer 1985	4-
Mouse	12 wk 2 d/wk		47			Boutwell and Bosch 1959	4-
Mouse	12 wk 2 d/wk		47			Boutwell and Bosch 1959	2-
Systemic							
Rat	120 d	Resp	250			Angerhofer 1985	4-
		Cardio	250				
		Gastro	250				
		Musc/sk	250				
		Hepatic	250				
		Renal	250				
		Derm/oc		50 (skin irritation)			

TABLE 2-3 (Continued)

Species	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Isomer
Developmental							
Rat	120 d		250			Angerhofer 1985	4-
Reproductive							
Rat	120 d		250			Angerhofer 1985	4-

Cardio = cardiovascular; d = day; Derm/oc = dermal/ocular; Gastro = gastrointestinal; hr = hour; LOAEL = lowest-observed-adverse-effect level; Musc/sk = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week; x = times

No studies were located regarding hematological effects in animals after dermal exposure to 4-nitrophenol.

Limited information is available regarding systemic effects in animals following dermal exposure to 4-nitrophenol. The highest NOAEL values and all reliable LOAEL values for each systemic effect are recorded in Table 2-l.

Respiratory Effects. No gross or histopathological alterations were observed in the lungs of rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Cardiovascular Effects. No gross or histological alterations in the heart or changes in heart weight were observed in rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Gastrointestinal Effects. No gross or histological alterations were seen in the gastrointestinal tract of rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Musculo/Skeletal. No gross or histological alterations were seen in skeletal muscles and bones of rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Hepatic Effects. No gross or histological alterations in the liver or changes in liver weight were observed in rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Renal Effects. No gross or histological alterations in the kidneys or changes in kidneys weight were seen in rats treated dermally with doses of 50-250 mg 4-nitrophenol/kg/day for 120 days (Angerhofer 1985).

Dermal/Ocular Effects. Moderate to severe cornea1 cloudiness, blistered conjunctival tissue, and cornea1 neovascularization were observed in rabbits after a single application of 27 mg of solid 4-nitrophenol/kg into the conjunctival sac (Monsanto 1983c). Only in one of six rabbits the effects appeared to be reversible during a 21-day observation period. Erythema and edema at the site of application were the most prevalent signs of exposure in rabbits when a saline suspension of 5,000 mg 4-nitrophenol was applied to the abraded dorsal surface for 24 hours (Monsanto 1983b). No adverse effects were noticed in the shaved dorsal surface of rabbits after application of 147 mg of dry solid 4-nitrophenol/kg for 4 hours (Monsanto 1984). However, when the solid 4-nitrophenol was applied moistened with saline, skin erythema and edema were observed. Skin scabbing and scarring were reported in rabbits 14 days after application of 181 mg 4-nitrophenol/kg moistened with saline for 24 hours (Monsanto 1983d). Partial recovery was observed by day 21. Application of 4-nitrophenol in daily doses of 50-250 mg 4-nitrophenol/kg to the skin of rats for 120 days resulted in dose-related dermal irritation

consisting of erythema, scaling, scabbing, and cracking of the skin (Angerhofer 1985). It is possible, however, that the solvent, ethanol, may have contributed to the development of these effects.

2.2.3.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals following dermal exposure to 2-nitrophenol or 4-nitrophenol.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals following dermal exposure to 2-nitrophenol or in humans following dermal exposure to 4-nitrophenol.

Application of 50-250 mg/kg/day of 4-nitrophenol to the skin of rats for 120 days had no effect on the weight or the gross and microscopic appearance of the brain (Angerhofer 1985). Information regarding the areas of the brain examined was not provided. However, since neurological tests were not performed, a reliable NOAEL for neurological effects cannot be determined.

2.2.3.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals following dermal exposure to 2-nitrophenol or in humans following dermal exposure to 4-nitrophenol.

In a 2-generation study, dermal application of 4-nitrophenol to rats in doses of 50-250 mg/kg/day for 120 days did not affect the appearance, behavior, or growth of the offspring (Angerhofer 1985). The NOAEL of 250 mg/kg is recorded in Table 2-3.

2.2.3.6 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals following dermal exposure to 2-nitrophenol or in humans following dermal exposure to 4-nitrophenol.

Reproductive performance was assessed in rats in a 2-generation study in which 4-nitrophenol was applied to the skin of the F_0 and F_1 generations in doses of 50-250 mg/kg/day for 120 days (Angerhofer 1985). Fertility (number of pregnancies/number mated), gestation (percentage of pregnancies resulting in birth of live litters), viability (pups surviving at least to day 4 of life), and lactation (pups surviving at least to day 21 of life) were unaffected by treatment with 4-nitrophenol. Histological examination of the reproductive organs of males and females revealed no treatment-related effects. The NOAEL of 250 mg/kg is recorded in Table 2-3.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following dermal exposure to 2-nitrophenol or 4-nitrophenol.

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans following dermal exposure to 2-nitrophenol or 4-nitrophenol.

Application of 2-nitrophenol or 4-nitrophenol (dissolved in dioxane) to the shaved backs of mice in doses of 47 mg nitrophenol/kg/day for 12 weeks did not induce skin tumors or lesions that could be considered precancerous in nature (Boutwell and Bosch 1959). These results should be interpreted with caution, since no other site was examined and the duration of the study may have been too short for evaluating carcinogenic potential.

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding the rate and extent of absorption in humans or animals following inhalation exposure to 2-nitrophenol or in humans after inhalation exposure to 4-nitrophenol.

Evidence of absorption of 4-nitrophenol by the inhalation route may be inferred from the fact that rats exposed to dusts of 4-nitrophenol (sodium salt) for 2 weeks developed adverse systemic effects (Smith et al. 1988).

2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans following oral exposure to 2-nitrophenol or 4-nitrophenol.

Indirect evidence of absorption of 2-nitrophenol and 4-nitrophenol has been presented in several animal studies. The sulfate conjugate of 4-nitrophenol was detected in the urine of rabbits after gavage administration of single doses between 182 and 264 mg/kg (Williams 1938). A similar finding was reported by Robinson et al. (1951a), who monitored the excretion of nitro compounds and conjugates in the urine of rabbits after gavage doses of both 2-nitrophenol (200-330 mg/kg) and 4-nitrophenol (150-200 mg/kg). Based on excretion data, it was apparent that at least 80%-90% of the dose was rapidly absorbed. In a monkey, oral absorption of 4-nitrophenol was fast since peak blood concentrations of the compound were achieved within minutes after a

gavage dose of 20 mg/kg (Lawford et al. 1954). The extent of absorption was not determined.

2.3.1.3 Dermal Exposure

No studies were located regarding absorption in humans or animals following dermal exposure to 2-nitrophenol or in humans following dermal exposure to 4-nitrophenol.

In animals, absorption efficiency appeared to be species-specific. In rabbits and beagle dogs, 35% and 11%, respectively, of a dose of ^{14}C-labeled 4-nitrophenol dissolved in ethanol and applied to the skin under a patch, was recovered in the urine over 7 days, indicating absorption through the skin (Snodgrass 1983). In the rabbits, the absorption rate was approximately 16% of the dose/day for 2 days, whereas in the dogs the absorption rate was 3% of the dose/day for 2 days. Thus, absorption was more extensive and more rapid in rabbits than in dogs. Unabsorbed 4-nitrophenol accounted for 53% and 86% of the applied dose in the rabbits and dogs, respectively (Snodgrass 1983).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals following inhalation exposure to 2-nitrophenol or 4-nitrophenol.

2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals following oral exposure to 2-nitrophenol or 4-nitrophenol.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals following dermal exposure to 2-nitrophenol or in humans following exposure to 4-nitrophenol.

Application of ^{14}C-labeled 4-nitrophenol to the skin of rabbits (0.12 mg/kg) and dogs (0.06 mg/kg) resulted in no detectable radioactivity in specimens of all major tissues and organs 7 days later (Snodgrass 1983). No attempt was made to determine distribution at an earlier time following exposure.

2.3.2.4 Other Routes of Exposure

Intravenous injection of ^{14}C-labeled 4-nitrophenol to rabbits (0.12 mg/kg) or dogs (0.06 mg/kg) resulted in undetectable levels of radioactivity in all major tissues and organs 7 days after treatment (Snodgrass 1983). No

attempt was made to determine distribution at an earlier time. The study by Snodgrass (1983) suggests that following dermal or parenteral exposure, 4-nitrophenol does not bioaccumulate.

2.3.3 Metabolism

No studies were located regarding metabolism in humans following inhalation, oral, or dermal exposure to 2-nitrophenol or 4-nitrophenol. Other data, extracted from studies with cultured human cells and perfused human tissues in vitro, are discussed below.

The major metabolic route for 2-nitrophenol and 4-nitrophenol is conjugation, with the resultant formation of either glucuronide or sulfate conjugates. Conjugates are more polar than the parent compounds and, therefore, are easier to excrete in the urine. Other possible routes of metabolism include reduction to amino compounds or oxidation to dihydric nitrophenols (catechols). In humans, the evidence is indirect and comes from studies of exposure to the pesticide parathion, of which 4-nitrophenol is a metabolite (Fatiadi 1984).

The metabolism of 2-nitrophenol and 4-nitrophenol in rabbits was studied by Robinson et al. (1951a), who showed that, with oral doses of 200-300 mg/kg, conjugation with glucuronic and sulfuric acids was almost complete. With both isomers, the major conjugation product excreted in the urine was nitrophenyl-glucuronide, accounting for approximately 70% of the dose: The corresponding sulfate conjugates were also excreted. Slight reduction to amino compounds occurred, 15% of the dose for the 4-isomer and 2%-3% for the 2-isomer. Oxidation products were also found in the urine; less than 1% of the 4-nitrophenol dose was oxidized to 4-nitrocatechol, whereas less than 1% of the 2-nitrophenol dose was detected as nitroquinone.

Similar results have been obtained in rats after intravenous administration of 4-nitrophenol (Machida et al. 1982). The glucuronide and sulfate conjugates could be detected in the plasma within 1 minute after the injection of doses between 1.6 and 8.0 mg/kg. Machida et al. (1982) also demonstrated that rat liver homogenates had the greatest amount of glucuronidation activity, followed by the kidney, lung, and small intestine homogenates, in decreasing order. Sulfation, however, was detected almost exclusively in the liver. No differences in conjugation mechanisms for 4-nitrophenol between male and female rats have been reported (Meerman et al. 1987).

The metabolism of 4-nitrophenol has also been studied in perfused organ preparations. Perfusion of human kidneys (isolated from cadavers) with 4-nitrophenol resulted in the formation of the glucuronide and sulfate conjugates (Diamond et al. 1982). Sulfate conjugates were found predominantly in mice livers perfused with low concentrations (4 µM) of 4-nitrophenol (Sultatos and Minor 1985). However, as the concentration of 4-nitrophenol was

increased, unchanged 4-nitrophenol and the glucuronide appeared in the effluent, indicating the presence of saturation kinetics. In perfused rat livers, three factors appeared to act as rate-determining for conjugation of 4-nitrophenol: concentration of 4-nitrophenol, supply of uridine diphosphate-glucuronic acid from carbohydrates for glucuronyltransferase, and activity of the enzyme (Reinke et al. 1981). Furthermore, the extent of liver conjugation in the rat was found to be modulated by the sympathetic nervous system through the hepatic nerves (Beuers et al. 1986).

Conjugation of 4-nitrophenol also occurred in cultured skin epithelial cells from humans (Rugstad and Dybing 1975), in isolated rat hepatocytes (Araya et al. 1986; Moldeus et al. 1976; Tonda and Hirata 1983), and in microsomes isolated from dog livers (Nakano et al. 1986).

Schemes of tentative metabolic pathways for 2-nitrophenol and 4-nitrophenol are presented in Figures 2-3 and 2-4, respectively.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals following inhalation exposure to 2-nitrophenol or 4-nitrophenol.

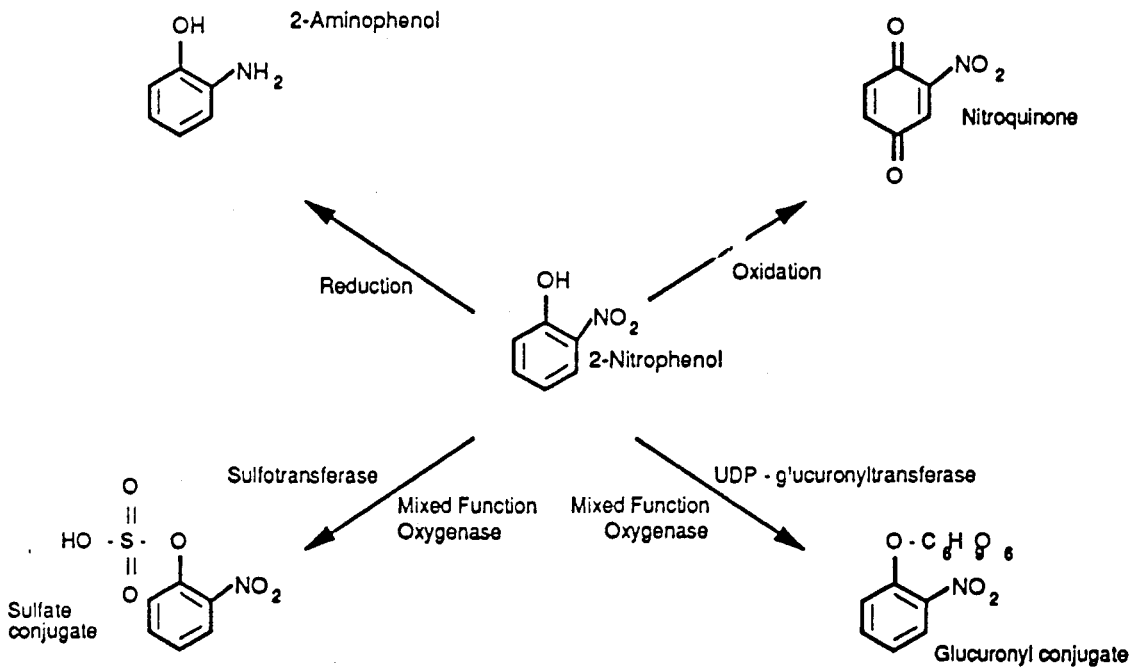
2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans following oral exposure to 2-nitrophenol or 4-nitrophenol.

As part of a study to compare the extent of sulfonation between phenol and substituted phenols, Williams (1938) reported that administration of doses between 182 and 264 mg/kg of 4-nitrophenol by gavage to rabbits resulted in excretion of approximately 25% of the dose in the urine as sulfate conjugate in less than a week. This finding was later confirmed by Robinson et al. (1951a), who showed that a dose of 150-200 mg 4-nitrophenol/kg given to rabbits was excreted in the urine, 70% as glucuronide and 12-20% as ethereal sulfate. In another experimental series, Robinson et al. (1951a) showed that in rabbits the urinary excretion of nitro compounds is almost complete in 1 day after oral administration of a dose of 200 mg/kg of 4-nitrophenol by gavage. The unchanged nitro group accounted for nearly 90% of the dose, whereas approximately 15% was reduced to amino compounds. In another series, Robinson et al. (1951a) found that less than 1% of a dose of 250 mg/kg of 4-nitrophenol was excreted in the urine oxidized to 4-nitrocatechol.

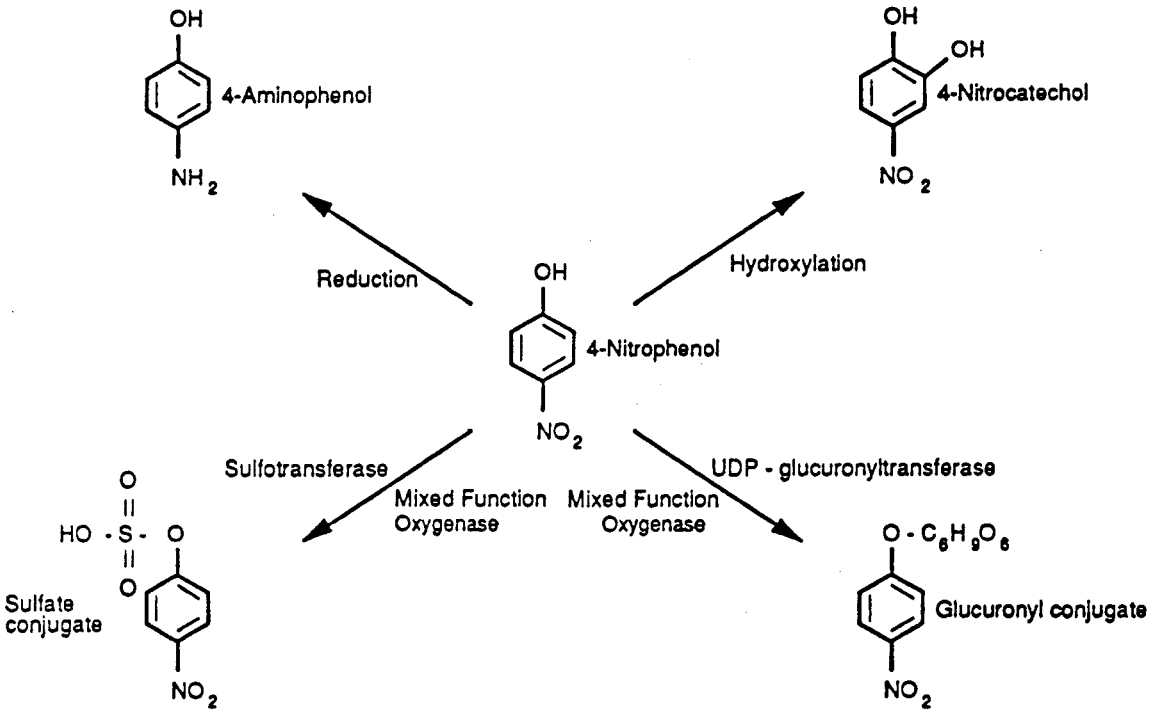
Using the same experimental protocols, Robinson et al. (1951a) demonstrated that when the rabbits were given 2-nitrophenol, the unchanged nitro group accounted for approximately 80% of the dose and 2-3% was detected as amino compounds. Nearly 70% of the dose was excreted as glucuronide and

FIGURE 2-3. Proposed Metabolic Pathway for 2-Nitrophenol*



*Adapted from Robinson et al. 1951a

FIGURE 2-4. Proposed Metabolic Pathway for 4-Nitrophenol*



*Adapted from Robinson et al. 1951a

10% as ethereal sulfate. Less than 1% was found oxidized to nitroquinone. The rapid elimination of nitrophenols may be due to the formation of conjugates, which, by being more polar than the parent compounds, are readily excreted in the urine.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals following dermal exposure to 2-nitrophenol or humans following dermal exposure to 4-nitrophenol.

Dermal application of ^{14}C-labeled 4-nitrophenol to dogs resulted in 11% of the dose (radioactive label) excreted in the urine over a period of 7 days. Fecal elimination was negligible. In rabbits, 78% of an absorbed dermal dose of ^{14}C-labeled 4-nitrophenol appeared in the urine in 1 day. As in dogs, fecal elimination accounted for less than 1% of the absorbed dose (Snodgrass 1983).

2.3.4.4. Other Routes of Exposure

Rats injected intravenously with a dose of 8.3 mg/kg of 4-nitrophenol excreted 35% of the dose as sulfate conjugate and 40% as glucuronide over a period of 24 hours (Meerman et al. 1987). No differences were noticed between males and females. Dogs given an intravenous dose (0.06 mg/kg) of ^{14}C-labeled 4-nitrophenol excreted 92% of the dose (labeled C) in the urine in the first day (Snodgrass 1983). Radioactivity in the feces accounted for approximately 1% over a 7-day period. Snodgrass (1983) used the same protocol in rabbits and found that 78% of the dose (0.12 mg/kg) was recovered in the urine within day 1; excretion was essentially complete by day 4. Fecal elimination accounted for less than 1% of the dose.

2.4 RELEVANCE TO PUBLIC HEALTH

No information was located regarding the effects of 2-nitrophenol or 4-nitrophenol in humans after inhalation, oral, or dermal exposure. The only toxicological signs of probable relevance are hematological effects observed in animals exposed to 2-nitrophenol or 4-nitrophenol. These effects were reported in an acute duration inhalation study. Limited longer-term inhalation and oral data were available for 4-nitrophenol. Oral lethal doses for the two isomers have been identified. From acute lethality studies, it appears that 2-nitrophenol is less toxic than 4-nitrophenol, but little additional information was available regarding the 2-isomer. Aside from the hematological effects, no other specific systems or organs have been identified as targets for 2-nitrophenol or 4-nitrophenol. Dermal and ocular effects of 4-nitrophenol have been identified, but are most likely nonspecific irritation. Since no human data were available, the relevance to public health of the effects observed in animals is not known. Studies that examined the effects of nitrophenols in animals used exposure levels that are several

orders of magnitude higher than those at which humans will be generally exposed.

Lack of adequate data precluded the derivation of an MRL for acute inhalation exposure to 4-nitrophenol. A concentration-related increase in methemoglobin was reported in rats in the acute inhalation study by Smith et al. (1988). However, inconsistencies in the values obtained in two different experimental series, the unknown toxicological significance of the methemoglobin increase, and the preliminary nature of the report were factors that greatly diminished the power of the study. Although supporting studies with 4-nitrophenol in other species were not located, nitroaromatic compounds are known inducers of methemoglobin both in humans and animals (Beard and Noe 1981; Ellenhorn and Barceloux 1988). An MRL for intermediate-duration inhalation exposure to 4-nitrophenol was not derived due to inconsistent methemoglobin values among the various subgroups of rats in the Hazleton (1983) study. Furthermore, methemoglobin was not monitored at terminal sacrifice (after 20 exposures). Since methemoglobin formation appears to be the most sensitive end point affected by 4-nitrophenol (Smith et al. 1988), other end points examined in this study were not selected for derivation of an intermediate-duration inhalation MRL. MRLs for chronic-duration inhalation exposure for 4-nitrophenol, or for any inhalation exposure duration for 2-nitrophenol are precluded by the lack of data. MRLs for acute- and chronic-duration oral exposure to 2-nitrophenol and 4-nitrophenol, and for intermediate-duration oral exposure to 2-nitrophenol could not be derived due to lack of data. Results from the study by Hazleton (1989) were not used for derivation of an MRL for intermediate-duration oral exposure to 4-nitrophenol due to uncertainty regarding the monitoring of methemoglobin. In this study, unexposed rats had unusually high methemoglobin values, suggesting that problems existed with the analytical method used. Increased methemoglobin was the most sensitive end point in rats exposed to 4-nitrophenol for 2 weeks (Smith et al. 1988). Acute-duration, intermediate-duration, and chronic-duration dermal MRLs were not derived for 2-nitrophenol or 4-nitrophenol due to the lack of an appropriate methodology for the development of dermal MRLs.

Death. No information regarding human fatalities due to inhalation, oral, or dermal exposure to 2-nitrophenol or 4-nitrophenol was located in the literature. Concentrations and doses causing death in animals have been reported for acute oral exposure to 2-nitrophenol and 4-nitrophenol, subchronic oral exposure to 4-nitrophenol, and acute dermal exposure to 4-nitrophenol. The cause of death was not reported. Acute oral toxicity data (LD_{5O}) reveal that 4-nitrophenol is considerably more toxic than 2-nitrophenol. No reports of lethality related to inhalation exposure to either 2-nitrophenol or 4-nitrophenol were located. The available information on the lethality of the nitrophenols is insufficient to assess the relevance to human health.

2. HEALTH EFFECTS

Systemic Effects. No studies were located regarding systemic effects in humans after inhalation, oral, or dermal exposure to 2-nitrophenol or 4-nitrophenol.

Respiratory Effects. A decrease in absolute and relative lung weight was noted in rats exposed to 2,119 mg 4-nitrophenol dust/m^3 for two weeks (Smith et al. 1988). Since histological examination of the lungs failed to reveal any morphological damage, the significance of the weight change is unclear. The existing evidence suggests that the respiratory system is not a target for acute or intermediate inhalation exposure to 4-nitrophenol. Wheezing, dyspnea, and lung congestion reported in rats receiving 70 mg 4-nitrophenol/kg/day or more orally were most likely due to terminal hypoxia and not to a specific effect on the respiratory system (Hazleton 1983). This conclusion is supported by the fact that rats surviving until sacrifice (13 weeks) did not show gross or microscopical alterations in the respiratory tract. The available information on respiratory effects of 4-nitrophenol is insufficient to assess the relevance to human health.

Hematological Effects. A relevant hematological effect, observed in rats, is the induction of methemoglobinemia after acute inhalation exposure to 112 mg 4-nitrophenol dust/m^3 for 2 weeks (Smith et al. 1988). Although this finding was reported in only one study, it appears relevant because aromatic amino and nitro compounds are known for causing the formation of methemoglobin in humans and animals (Beard and Noe 1981). Inconsistent methemoglobin values arising from possible analytical problems precluded a reliable assessment of hematological effects of intermediate-duration exposure of rats to 4-nitrophenol dust (Hazleton 1983) or to oral doses of 4-nitrophenol in an intermediate-duration study (Hazleton 1989).

Hepatic Effects. Rats exposed to a dust of 4-nitrophenol at concentrations of 292 and 2,119 mg 4-nitrophenol/m^3 for 2 weeks had a slight increase in serum levels of SGOT (Smith et al. 1988). However, the significance of this effect is unclear. Furthermore, no histological evidence of liver damage was observed. Similar results were reported in rats exposed to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983) and in rats administered oral doses of up to 140 mg 4-nitrophenol/kg for 13 weeks (Hazleton 1989). The existing evidence indicates that the liver is not a target for 4-nitrophenol after acute- and intermediate-duration exposures. The available information regarding hepatic effects of 4-nitrophenol in animals is insufficient to assess the potential for hepatic effects in humans exposed to 2-nitrophenol or 4-nitrophenol.

Renal Effects. Proteinuria and darker urine were observed in rats that inhaled a dust of 4-nitrophenol at concentrations of 292 and 2,119 mg 4-nitrophenol/m^3 for 2 weeks (Smith et al. 1988). These findings could not be interpreted as unequivocal evidence of kidney damage since they can also be present under unrelated conditions. Furthermore, no histological alterations

were found in the kidneys. Similar findings were reported in rats exposed to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). Kidney congestion was reported in rats that died prematurely in a 13-week gavage study (Hazleton 1989), but this effect was most likely caused by terminal hypoxia, since rats that survived did not exhibit kidney lesions at sacrifice. The available information regarding renal effects of 4-nitrophenol in animals is insufficient to assess the potential for renal effects in humans exposed to 2-nitrophenol or 4-nitrophenol.

Dermal/Ocular Effects. Two studies were located that described dermal/ocular effects in rats after inhalation exposure. In one study, rats exposed to 4,033 mg 4-nitrophenol dust/m^3 for 4 hours developed cornea1 opacity (Smith et al. 1988). The second study reported anterior capsular cataracts in rats exposed to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983). Cornea1 opacity was also reported in rabbits after a single local application of 27 mg 4-nitrophenol/kg (Monsanto 1983c). It is, therefore, possible that the effect seen in the Smith et al. (1988) study was caused by direct contact of the 4-nitrophenol dusts with the cornea rather than by inhalation of the 4-nitrophenol. Similarly, cataracts reported by Hazleton (1983) are likely to have been caused by direct contact with 4-nitrophenol; however, a systematic effect cannot be totally excluded. Application of single doses of 147 mg 4-nitrophenol or more to the skin of rabbits (Monsanto 1984), or of 50 mg 4-nitrophenol/kg/day or more for 120 days to the skin of rats (Angerhofer 1985) resulted in skin irritation. It is important to point out that 4-nitrophenol was much more toxic to the skin when applied moistened with saline than when the dry solid was used. The evidence available suggests that 4-nitrophenol may cause dermal and eye irritation when applied locally in humans.

Neurological Effects. No information was identified regarding neurological effects in humans or animals following exposure to 2-nitrophenol or in humans following exposure to 4-nitrophenol. Inhalation exposure of rats to 2,119 mg 4-nitrophenol dust/m^3 (sodium salt) for 2 weeks (Smith et al. 1988) or to 30 mg 4-nitrophenol dust/m^3 for 4 weeks (Hazleton 1983) did not affect brain weight or the gross or histological appearance of the central and peripheral nervous system. Similar lack of effects were reported in rats administered oral doses of 140 mg 4-nitrophenol/kg/day for 13 weeks (Hazleton 1989). It must be mentioned, however, that none of these studies conducted tests for neurological function. The available information is insufficient to assess the potential for neurological effects in humans exposed to 2-nitrophenol or 4-nitrophenol.

Developmental Effects. No studies were located that examined the developmental effects of 2-nitrophenol in humans or animals or 4-nitrophenol in humans. In a 3-generation study, dermal application of 4-nitrophenol to rats, in doses of 50-250 mg/kg for 120 days that included the gestation period, did not affect the appearance, behavior or growth of the offspring

(Angerhofer 1985). Oral administration of 400 mg/kg of 4-nitrophenol to mice during gestation did not alter the reproductive index, a measure of prenatal death (Plasterer et al. 1985). However, in the latter study, the teratogenic potential of 4-nitrophenol could not be dismissed. In the absence of further information, no inference regarding possible effects in humans can be made.

Reproductive Effects. It is not known whether 2-nitrophenol or 4-nitrophenol could cause reproductive effects in humans. Rats exposed to 30 mg 4-nitrophenol/m^3 for 4 weeks (Hazleton 1983) or administered 140 mg 4-nitrophenol/kg/day for 13 weeks (Hazleton 1989) had no treatment-related effects on the weight or histopathology of the reproductive organs, but reproductive performance was not assessed. In a 2-generation study in rats, dermal application of 4-nitrophenol in doses of 50-250 mg/kg for 120 days did not alter reproductive performance. The relevance of this information to human health is not known. Data regarding the reproductive effects of 2-nitrophenol were not available.

Genotoxic Effects. No studies were located regarding the genotoxic effects of 2-nitrophenol or 4-nitrophenol in humans or animals by inhalation, oral, or dermal routes. 4-Nitrophenol was not mutagenic in vivo as judged by the dominant lethal assay and the host-mediated assay in mice (Buselmaier et al. 1973). No information was available regarding mutagenicity of 2-nitrophenol in vivo.

As indicated in Table 2-4, 2-nitrophenol did not increase the frequency of reverse mutations in Salmonella typhimurium or in Escherichia coli in the presence or absence of metabolic activation, nor did it induce DNA damage when tested in Bacillus subtilis. No data were available regarding genotoxic properties of 2-nitrophenol in eukaryotic organisms.

The in vitro genotoxicity of 4-nitrophenol has been investigated in prokaryotic organisms and in mammalian cell systems. The overall evidence indicates that 4-nitrophenol is not mutagenic in the presence or absence of activating systems in S. typhimurium and E. coli (Table 2-5). One positive result was reported by Shimizu and Yano (1986), who showed that 4-nitrophenol induced DNA damage when tested in B. subtilis by the ret assay. According to the authors (Shimizu and Yano 1986), this assay appears to be more sensitive for nitro compounds in general than the standard Ames Test. Weaker genotoxic effects were reported in two studies (Adler et al. 1976; Garrett and Lewtas 1983). The hypothesis that reduction of the nitro group is required to observe mutagenic effects was tested by Dellarco and Prival (1989). These authors did not observe an increase in mutagenicity when 2-nitrophenol or 4-nitrophenol was incubated in the presence of S-9 and flavin mononucleotide mixture in S. typhimurium. 4-Nitrophenol was not mutagenic when tested in mammalian cells with or without metabolic activation. The In vitro and in vivo information, negative data or lack of data, respectively, would suggest that 2-nitrophenol or 4-nitrophenol does not pose a genotoxic threat to humans.

TABLE 2-4. Genotoxicity of 2-Nitrophenol *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation)	Gene mutation	No data	-	Chiu et al. 1978
S. typhimurium (plate incorporation)	Gene mutation	-	-	Suzuki et al. 1983
S. typhimurium (plate incorporation)	Gene mutation	-	-	Dellarco and Prival 1989
S. typhimurium (plate incorporation)	Gene mutation	-	-	Shimizu and Yano 1986
Escherichia coli sd-4-73 (spot test)	Gene mutation	No data	-	Szybalski 1958
Bacillus subtilis (plate incorporation)	DNA damage	No data	-	Shimizu and Yano 1986

- = negative result

TABLE 2-5. Genotoxicity of 4-Nitrophenol In Vitro

Species (test system)	End point	Result: With activation	Result: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (plate incorporation)	Gene mutation	-	-	Suzuki et al. 1983
S. typhimurium (plate incorporation)	Gene mutation	-	-	Probst et al. 1981
S. typhimurium (plate incorporation)	Gene mutation	-	-	Haworth et al. 1983
S. typhimurium (plate incorporation)	Gene mutation	-	-	Shimizu and Yano 1986
S. typhimurium (plate incorporation)	Gene mutation	-	-	Dellarco and Prival 1989
Escherichia coli (plate incorporation)	Gene mutation	-	-	Probst et al. 1981
E. coli (spot test)	Gene mutation	-	-	Syzbalski 1958
E. coli (plate incorporation)	Prophage induction	-	No data	Ho and Ho 1981
Proteus mirabilis (plate incorporation)	DNA damage	No data	(+)	Adler et al. 1976
E.coli (disc assay)	DNA repair	No data	-	Rashid and Mumma 1986
S. typhimurium (disc assay)	DNA repair	No data	-	Rashid and Mumma 1986
Bacillus subtilis (plate incorporation)	DNA damage	No data	+	Shimizu and Yano 1986
Mammalian cells:				
Rat hepatocytes (culture)	DNA repair	No data	-	Probst et al. 1981
Mouse lymphoma cells	Forward mutation	-	-	Oberly et al. 1984
Mouse lymphoma cells	Forward mutation	-	No data	Amacher and Turner 1982
Chinese hamster ovary cells (culture)	Inhibition of DNA synthesis	No data	(+)	Garrett and Lewtas 1983

+ = positive result
- = negative result
(+) = weakly positive result

Cancer. No studies were located regarding the carcinogenic potential of 2-nitrophenol or 4-nitrophenol in humans by any route of exposure or in animals by the inhalation or oral route. Neither isomer induced tumors when applied to the backs of mice in doses of 47 mg/kg/day for 12 weeks (Boutwell and Bosch 1959). However, since no other site was examined and the duration of the study was only 12 weeks, the results should be interpreted with caution. The relevance of this information to human health is unknown. NTP (1991) recently conducted a review of a 2-year skin painting study with 4-nitrophenol in mice. The panel concluded that under the conditions of the study, there was no evidence of carcinogenic activity in male or female Swiss-Webster mice receiving doses of up to 160 mg 4-nitrophenol/kg for 78 weeks.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2-nitrophenol and 4-nitrophenol are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health

impairment (e.g., DNA adducts). Biomarkers of effects caused by 2-nitrophenol and 4-nitrophenol are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to 2-Nitrophenol and 4-Nitrophenol

No studies were located regarding levels of 2-nitrophenol or 4-nitrophenol in human tissues, fluids, or excreta that were associated with exposure to nitrophenols. To assess exposure to the pesticide parathion, of which 4-nitrophenol is a metabolite, several methods have been developed to monitor 4-nitrophenol in human urine (Arterberry et al. 1961; Fatiadi 1984). In general, it is agreed that these methods are not suitable indicators for studying the severity of the intoxication caused by parathion (or perhaps nitrophenols) or the appearance of toxic signs; rather, these methods indicate acute exposure to the pesticide (or nitrophenols) (Arterberry et al. 1961; Pena-Egido et al. 1988). The reason is that 2-nitrophenol and 4-nitrophenol conjugates are completely and rapidly excreted in the urine. Therefore, unless a very high dose is given, urinary levels will fall to near zero in a short time (48 hours). It is not known if urinary excretion of 2-nitrophenol or 4-nitrophenol (or their conjugates) can be associated quantitatively with exposure to these chemicals.

2.5.2 Biomarkers Used to Characterize Effects Caused by 2-Nitrophenol and 4-Nitrophenol

No toxic signs specific to 2-nitrophenol or 4-nitrophenol exposure have yet been identified. However, nitro aromatic and amino compounds in general are known to induce formation of methemoglobin in humans and experimental animals (Beard and Noe 1981). Although response varies considerably among species, it appears that 2-nitrophenol and 4-nitrophenol are not among the most potent methemoglobin inducers. Furthermore, methemoglobinemia can also be caused by inherited disorders and a number of drugs including sulfonamides and benzocaine.

2.6 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding interactions of 2-nitrophenol or 4-nitrophenol with other chemicals in vitro or regarding interactions of 2-nitrophenol with other chemicals in vivo. However, it was reported that, in ethanol-treated rats, 4-nitrophenol is rapidly metabolized to 4-nitrocatechol, which competes with 4-nitrophenol for the formation of sulfate and glucuronide conjugates (Reinke and Moyer 1985). This prevention of the conjugation of

4-nitrophenol may lead to the formation of amino derivatives, which can then induce methemoglobinemia.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Human populations that have experienced health effects from exposure to 2-nitrophenol or 4-nitrophenol have not been identified, but little research has been conducted on this subject. Based on results from animal studies, as described in Section 2.6, it is possible that individuals who consume ethanol may have slower rates of clearance of 4-nitrophenol. This subpopulation, if exposed to 4-nitrophenol, may be considered potentially susceptible. Furthermore, newborn infants utilize fetal hemoglobin, which has reduced oxygen-carrying capacity, and also have low levels of nicotinamide adenine dinucleotide diaphorase, which continuously reduces methemoglobin; therefore, infants (as well as individuals congenitally deficient in this enzyme) may represent unusually susceptible subpopulations. Data regarding health effects in humans exposed to 2-nitrophenol were not available.

2.8 MITIGATION OF EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to nitrophenols. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to nitrophenols. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.
No studies were located regarding health effects induced by nitrophenols in humans. However, studies in animals exposed to nitrophenols (Smith et al. 1988) and data regarding toxicity of other related compounds (nitrates/nitrites) in humans and animals (see ATSDR 1991) indicate that the major effect of absorption of high amounts of nitrophenols would be an increased formation of methemoglobin in red blood cells. Methemoglobin results from iron in the ferrous state being oxidized to the ferric state. Methemoglobin is unable to combine reversibly with oxygen and carbon dioxide and also causes a shift in the oxygen dissociation curve toward increased oxygen affinity, preventing the transfer of oxygen from the blood to the tissues. Clinical effects of methemoglobinemia are closely related to the percentage of methemoglobin in the blood (see ATSDR 1991). Concentrations up to 20% cause central cyanosis, but are usually asymptomatic. With higher methemoglobin concentrations, CNS depression (headache, dizziness, fatigue, lethargy) and dyspnea may develop. Methemoglobin levels over 45% lead to hypotension, cardiac arrhythmias, metabolic acidosis, and shock. Further CNS depression may cause convulsions, coma, and eventually death. Newborn infants are especially susceptible to methemoglobin induced effects (see Section 2.7).

In addition, ethanol consumers and individuals with certain enzyme deficiencies may be susceptible (see Section 2.6 and 2.7). The initial steps following removal of the individual from the exposure source are skincleansing, if dermal exposure is suspected. A caution should be employed with the administration of emetics (syrup of ipecac) in cases when ingestion of nitrophenols is suspected (Ellenhorn and Barceloux 1988; Stutz and Janusz 1988). Emesis has been suggested to be followed by administration of a suspension of activated charcoal in water to bind any toxicant remaining in the gastrointestinal tract. Subsequent steps have been aimed at chemically reducing methemoglobin back to oxyhemoglobin. A commonly used intervention for reducing methemoglobin is intravenous infusion of a solution of methylene blue (Ellenhorn and Barceloux 1988). Methylene blue acts as a cofactor to increase the chemical reduction of methemoglobin in the red blood cells in the presence of nicotinamide adenine dinucleotide (NADPH) (Ellenhorn and Barceloux 1988). Methylene blue is oxidized to leukomethylene blue, which donates electrons for the nonenzymatic reduction of methemoglobin to oxyhemoglobin. Administration of oxygen has been suggested in all cases of nitrophenols poisoning. In addition, standard control for convulsions and arrhythmias has been proposed. In life-threatening situations, hyperbaric oxygen therapy and blood transfusion have been recommended (see ATSDR 1991).

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) *to* assess whether adequate information *on* the health effects of 2-nitrophenol and 4-nitrophenol is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2-nitrophenol and 4-nitrophenol.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of 2-Nitrophenol and 4-Nitrophenol

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2-nitrophenol and 4-nitrophenol are summarized in Figures 2-5 and 2-6, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of 2-nitrophenol and 4-nitrophenol. Each dot in the figure indicates that one or

more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

As seen from Figures 2-5 and 2-6, no information is available regarding the health effects of either 2-nitrophenol or 4-nitrophenol in humans. The only information available regarding 2-nitrophenol is provided by two studies that determined the oral LD_{50} in rats and mice, and a dermal cancer study. Data are available in animals for lethality after inhalation, oral, or dermal exposure to 4-nitrophenol. One study reported effects of 4-nitrophenol after acute inhalation exposure; however, assessing the significance of most of the effects, such as immunological, neurological, and developmental (or Lack thereof), is difficult due to the incomplete examination of some end points. Data were available for systemic effects after oral exposure to 4-nitrophenol, and one pilot study examined developmental effects of this isomer. A limited number of dermal studies provided information concerning lethality, systemic effects after intermediate exposure, and developmental and reproductive effects of 4-nitrophenol. Information regarding the carcinogenicity of 4-nitrophenol was available from a single dermal study.

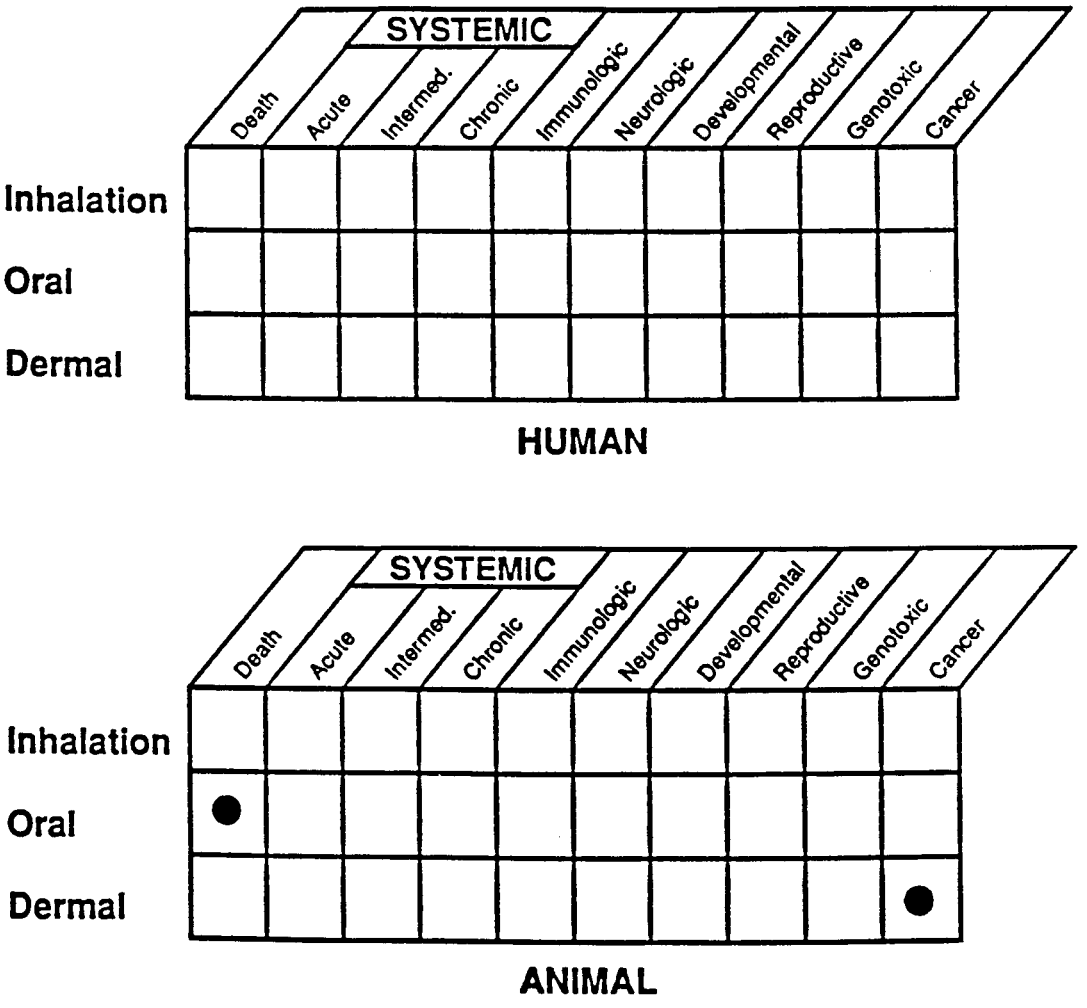
2.9.2 Data Needs

Acute-Duration Exposure. No data were Located indicating specific organs or systems as targets for 2-nitrophenol or 4-nitrophenol in humans by any route of exposure. However, amino and nitro aromatic compounds in general have been known to induce methemoglobinemia in humans (Beard and Noe 1981). The data in experimental animals were insufficient to derive oral and inhalation MRLs.

Information is lacking regarding the cause of death in the acute-duration studies, most of which have been conducted in rats. The significance of the renal effects, identified in the only acute-duration inhalation study available (Smith et al. 1988), could be clarified with a better designed acute inhalation study. Additional acute-duration studies by the oral and dermal routes would provide information on interspecies differences seen for dermal absorption and on the mechanisms of lethality, as well as on the thresholds for systemic toxicity due to acute-duration exposure for both 2-nitrophenol and 4-nitrophenol, particularly 2-nitrophenol.

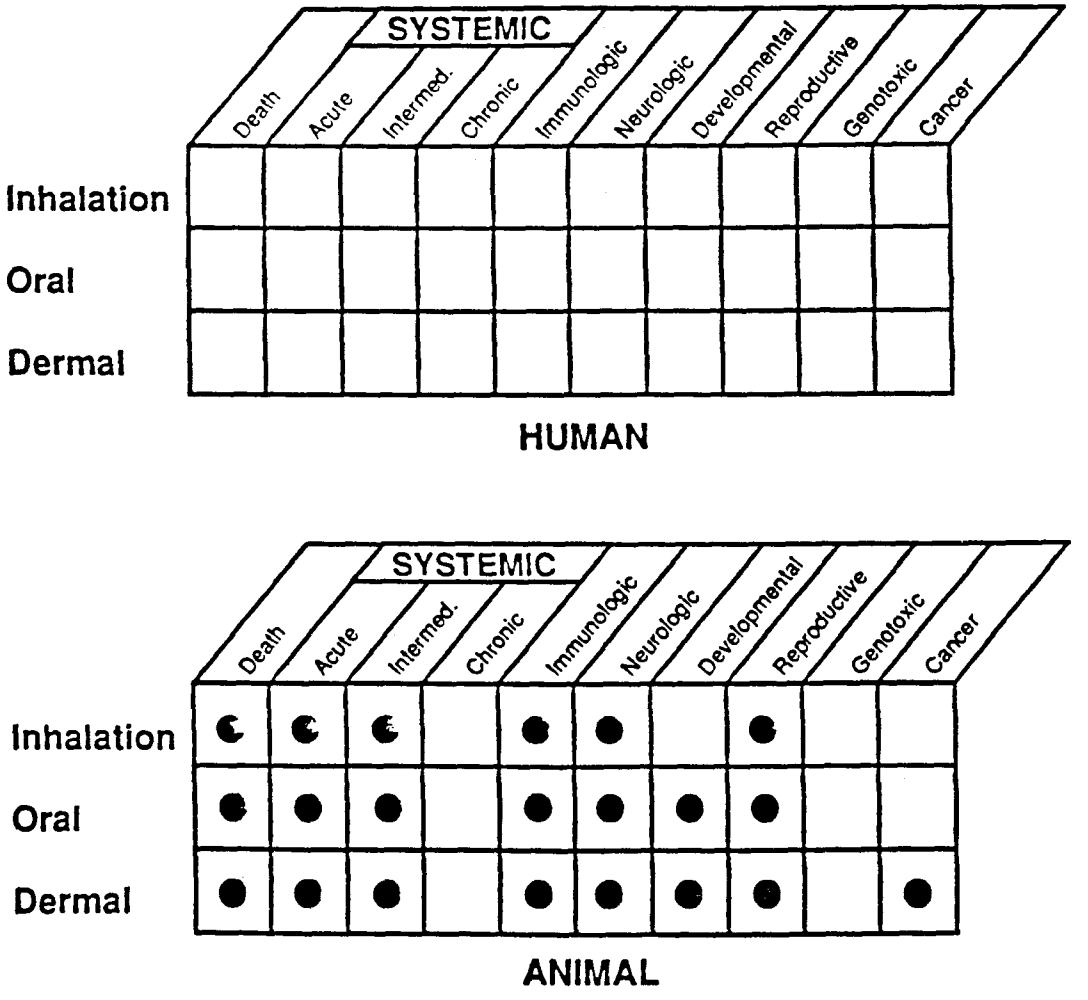
Careful dose-response studies on the effect of nitrophenols on the development of methemoglobinemia, in multiple species, both sexes, and at multiple doses, would provide information on an effect that is relevant to humans. Studies in rabbits could provide data on what appears to be the most sensitive species, as judged by data on acute lethality by the oral route (Williams 1938). The limited pharmacokinetic data do not suggest routespecific target organs. Because 2-nitrophenol and 4-nitrophenol are rapidly removed from the circulation and excreted (see Chapter 2.3), they will not

FIGURE 2-5. Existing Information on Health Effects of 2-Nitrophenol



● Existing Studies

FIGURE 2-6. Existing Information on Health Effects of 4-Nitrophenol



● Existing Studies

accumulate. This is particularly important in intermittent-exposure studies and in occupational settings and applies to intermediate- and chronic-duration studies, as well. However, additional studies that use continuous exposure would provide information relevant to potential exposure by populations surrounding hazardous waste sites.

Intermediate-Duration Exposure. No data were located that identified target organs in humans following intermediate-duration exposure to 2-nitrophenol or 4-nitrophenol by any route. An intermediate-duration study by the dermal route was conducted in rats, but some of the effects could be attributed to the vehicle used (Angerhofer 1985). Therefore, a dermal study with different vehicles would provide information on the effects that can be attributed to 4-nitrophenol and those attributed to the solvents used. Intermediate-duration studies by the inhalation (Hazleton 1983) and oral (Hazleton 1989) routes were conducted in rats. However, these studies could not define reliable NOAELs and LOAELs for methemoglobin formation, a sensitive end point in rats, due to analytical problems. Repeating these studies would eliminate the uncertainty regarding the threshold-effect level for this and other end points. There are no pharmacokinetic data that would suggest route-specific target organs. No data were available in animals regarding 2-nitrophenol. Intermediate-duration exposure studies with 2-nitrophenol would provide information on the thresholds for systemic toxicity for this isomer, as well as information regarding reproductive effects. Intermediate-duration studies in other species might provide information that could be relevant to human exposure, especially populations surrounding hazardous waste sites where humans might be exposed for similar durations.

Chronic-Duration Exposure and Cancer. Chronic inhalation, oral, or dermal studies are not available for either 2-nitrophenol or 4-nitrophenol. Studies using well-designed experiments using complete dose and time protocols, and measuring all sensitive toxicological end points, would provide information on the health effects associated with long-term exposure to 2-nitrophenol and 4-nitrophenol. These studies could provide information on subtle toxicological changes in organs associated with long-term exposure to low levels of 2-nitrophenol or 4-nitrophenol. No pharmacokinetic data suggest route-specific target organs. Inhalation and dermal studies are particularly relevant to individuals in occupational settings and to populations surrounding hazardous waste sites where humans might be exposed for similar durations.

No data were located regarding the carcinogenic potential of 2-nitrophenol or 4-nitrophenol in humans exposed by the inhalation, oral, or dermal route. A single dermal study assessed the carcinogenicity of 2-nitrophenol and 4-nitrophenol in mice (Boutwell and Bosch 1959). However, in this study, mice were exposed to only one dose level of 2-nitrophenol or 4-nitrophenol, and the duration of the study was inappropriate. In addition, only the site where the chemicals were applied was examined. Since

4-nitrophenol is a metabolite of the pesticide parathion, a chronic-duration study by the oral (diet and drinking water) and inhalation routes would provide information relevant to possible exposure in humans. Furthermore, since it is generally agreed that reduction of the nitro group to the amino group transforms the molecule into a more reactive (more electrophilic) one, studies with the reduced nitrophenols would provide information on the potential carcinogenicity of the metabolites. However, according to pharmacokinetic data, the extent of reduction of nitrophenols to amino compounds is minimal. NTP (1991) conducted a 2-year skin-painting study with 4-nitrophenol in mice; after reviewing the report, a peer review panel concluded that under the conditions of the study, there was no evidence of carcinogenic activity in male or female Swiss-Webster mice receiving doses of up to 160 mg 4-nitrophenol/kg 3 times/week for 78 weeks (see Section 2.9.3).

Genotoxicity. No studies were identified that evaluated genotoxic effects in humans or animals following inhalation, oral, or dermal exposure to 2-nitrophenol or 4-nitrophenol. Several In vitro studies suggest that 2-nitrophenol is not mutagenic in bacterial systems (Table 2-4); therefore, additional In vitro studies would add little to the database. No studies were identified regarding genotoxicity of 2-nitrophenol in eukaryotic organisms and mammalian cells. Such studies would provide information regarding the genotoxicity of 2-nitrophenol in those systems.

The available In vitro genotoxicity studies regarding 4-nitrophenol indicate that this isomer is not mutagenic in bacterial systems or in mammalian cells (Table 2-5). Further studies using the CHO/HGPRT mutation assay would help interpret the weak positive result reported by Garrett and Lewtas (1983) with this assay. Studies in eukaryotic organisms would certainly complement the existing information in prokaryotes.

Reproductive Toxicity. No data on the effects of 2-nitrophenol on the reproductive system for any time period or route of exposure are available. Such data, if available, would provide information on the potential toxic effects of 2-nitrophenol on the reproductive system of animals, information which, in turn, may be relevant to humans. The effects of 4-nitrophenol on reproductive performance have been examined in rats treated dermally (Angerhofer 1985). This study found no adverse effects. Available pharmacokinetic data do not suggest route-specific target organs. Studies were available that examined the subchronic effects of 4-nitrophenol on the gross and histological appearance of reproductive organs in rats after inhalation (Hazleton 1983) and oral (Hazleton 1989) exposure. However, reproductive performance was not assessed in these studies, therefore, a multigeneration study by the oral and inhalation routes would add information that could be relevant to humans.

Developmental Toxicity. No information was available indicating that 2-nitrophenol affects development in humans or animals following inhalation,

oral, or dermal exposure. The data regarding 4-nitrophenol were limited to one pilot study in which no gross abnormalities were observed in the offspring of mice dosed orally during pregnancy (Plasterer et al. 1985). In this study, however, a complete examination of the pups for possible teratogenic effects was not performed. It is not known whether 2-nitrophenol or 4-nitrophenol crosses the placenta, but the low molecular weight suggests that it (or its metabolites) does. Available pharmacokinetic data do not suggest routespecific target organs. Developmental studies in mammals treated orally, dermally, or by inhalation would provide information on possible fetotoxic and teratogenic effects that might be relevant to humans.

Immunotoxicity. No information was available indicating that, in humans or animals, the immune system is a target for either 2-nitrophenol or 4-nitrophenol. No histopathological effects were observed in organs and tissues involved in immunological functions of rats exposed by inhalation to 4-nitrophenol for 2 weeks (Smith et al. 1988) or 4 weeks (Hazleton 1983). Similar lack of effects was reported in rats treated dermally with 4-nitrophenol in an intermediate-duration study (Angerhofer 1985) or in rats administered 4-nitrophenol orally for 13 weeks (Hazleton 1989). However, none of these studies conducted tests for immunocompetence. In general, the immune system does not appear to be a target for nitro aromatic compounds. Dermal sensitization studies in animals might provide information on whether 2-nitrophenol or 4-nitrophenol are likely to cause an allergic response.

Neurotoxicity. No studies were located regarding the neurotoxic effects of 2-nitrophenol or 4-nitrophenol in humans, by any route of exposure. The limited data available in animals suggest that the nervous system is not a target for either 2-nitrophenol or 4-nitrophenol. No histopathological effects were observed in the central or peripheral nervous system of rats exposed by inhalation to 4-nitrophenol for 2 weeks (Smith et al. 1988) or 4 weeks (Hazleton 1983). Similar negative findings were reported in rats after subchronic dermal treatment with 4-nitrophenol (Angerhofer 1985) and in rats administered 4-nitrophenol orally for 13 weeks (Hazleton 1989). However, none of these studies tested neurological functions. Available pharmacokinetic data do not suggest route-specific target organs. Studies in other species and by the oral route of exposure, as well as tests for neurological impairment in animals, might provide information that could be relevant to humans.

Epidemiological and Human Dosimetry Studies. Health effects from humans exposed to 2-nitrophenol or 4-nitrophenol have not been reported. As discussed in Chapter 5, the potential for environmental exposure to 2-nitrophenol or 4-nitrophenol is considered low, although individuals living near waste sites where 2-nitrophenol and 4-nitrophenol have been identified represent a subpopulation with potential exposure to these chemicals. Moreover, individuals involved in the manufacture or processing of 2-nitrophenol and 4-nitrophenol clearly represent a potentially exposed

subpopulation. Epidemiology studies of people living in areas where nitrophenol has been detected in ambient and drinking water, near industries releasing nitrophenols, or near hazardous waste sites and of people occupationally exposed could provide information on whether nitrophenols produce effects in humans similar to those seen in animals or produce other toxic effects.

Biomarkers of Exposure and Effect. Information regarding populations exposed specifically to 2-nitrophenol or 4-nitrophenol is not available. However, data derived from animal studies indicate that unchanged 2-nitrophenol or 4-nitrophenol or the sulfate and/or glucuronide conjugates monitored in the urine represent biomarkers of exposure. The same would probably occur in humans. This assumption is based on studies in populations exposed to the pesticide parathion, of which 4-nitrophenol is a metabolite. Individuals exposed to parathion excreted 4-nitrophenol and conjugates in the urine (Fatiadi 1984). G-Nitrophenol is also a metabolite of pesticides other than parathion.(Fatiadi 1984) and of nitrobenzene (Piotrowski 1967; Robinson et al. 1951b). However, because 2-nitrophenol and 4-nitrophenol and their metabolites are rapidly excreted in the urine, these biomarkers are only valuable in evaluating acute situations, as demonstrated by Arterberry et al. (1961) in humans exposed to parathion. Hence, the development of methods to detect alternative biomarkers, the presence of which in body fluid or tissues can be associated with chronic exposure levels of nitrophenol, would be useful.

Information regarding populations exposed specifically to 2-nitrophenol or 4-nitrophenol is not available. Consequently, no specific alteration has been identified. Nonetheless, nitro aromatic and amino compounds in general induce formation of methemoglobin in humans and experimental animals (Beard and Noe 1981). However, it appears that 2-nitrophenol and 4-nitrophenol are not among the most potent methemoglobin inducers. In humans, methemoglobin in blood must reach a level of approximately 40% (normal levels are less than 1%) for serious symptoms such as cyanosis, coma, or stupor, to appear; these blood levels of methemoglobin are reached only with very high doses of nitro compounds. Therefore, identification of signs and symptoms associated with low levels of exposure would aid in the early detection of exposure. Furthermore, methemoglobinemia can also be caused by inherited disorders and a number of drugs, including sulfonamides and benzocaine.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding absorption, distribution, metabolism, or excretion of 2-nitrophenol or 4-nitrophenol in humans by any route of exposure, or in animals by the inhalation route. Indirect evidence indicates that absorption of 2-nitrophenol and 4-nitrophenol by the oral route is fast and almost complete (Robinson et al. 1951a). However, only 35% and 11% of a dermally applied dose of 4-nitrophenol was absorbed in rabbits and dogs, respectively, over a 7-day period (Snodgrass 1983). Limited data regarding distribution

showed that dermal or intravenous dosing of 4-nitrophenol to rabbits and dogs results in undetectable amounts of the chemical in major organs and tissues 7 days after dosing (Snodgrass 1983). Examination of the distribution at earlier times could provide important information regarding possible target organs and tissues. Data regarding 2-nitrophenol were not available. Although the metabolism of 2-nitrophenol and 4-nitrophenol has been examined only after oral dosing, a number of In vitro studies support the findings obtained in vivo. The excretion of 2-nitrophenol and 4-nitrophenol has been quantitated after oral, dermal, and intravenous dosing. Studies in which a range of doses are applied would provide information regarding possible saturation phenomena.

Comparative Toxicokinetics. Data were not available regarding the toxicokinetics of 2-nitrophenol or 4-nitrophenol in humans. In vivo toxicokinetic studies have been performed in rabbits (oral and dermal routes) and dogs (dermal route), with qualitatively similar results regarding absorption rates, metabolic pathways, and excretion rates (Robinson et al. 1951a, Snodgrass 1983; Williams 1938). These studies, however, have used single doses; therefore, it is not known if the similarities would persist over a range of doses. The limited size of the database precludes the identification of an animal species that could serve as the best model for extrapolating results to humans. Data obtained in humans after exposure to the pesticide parathion, of which 4-nitrophenol is a metabolite, suggest that conjugation is also the predominant metabolic route, and that 4-nitrophenol is also rapidly excreted in the urine, but quantitative data are not available. Due to the lower expense and wider usage of rats and mice, these should do well as study species, unless other ones are shown to be of more interest. Once reliable end points are determined in other species, it should be important to verify that primates are affected in a similar manner, in order to ensure that no unforeseen health effect might occur in humans.

Mitigation of Effects. The most prevalent sign of nitrophenol poisoning is increased formation of methemoglobin (Ellenhorn and Barceloux 1988). The most widely used antidote for treating methemoglobinemia is methylene blue, although other reducing agents such as ascorbic acid have been used with questionable results. Therefore, studies identifying alternate antidotes for the treatment of methemoglobinemia would be useful in providing a therapeutic choice for mitigation of this adverse effect. This is particularly relevant in view of the fact that methylene blue is poorly absorbed from the gastrointestinal tract and high intravenous doses produce unwanted side effects (Ellenhorn and Barceloux 1988).

2.9.3 On-going Studies

NTP (1991) conducted an 18-month skin-painting study with 4-nitrophenol in Swiss-Webster mice. In this study, 4-nitrophenol in acetone was applied to the interscapular skin of mice at concentrations of 0, 40, 80, and 160 mg/kg

3 days/week for 78 weeks. Administration of 4-nitrophenol did not affect body weight gain. Starting at week 60, high mortality occurred in all groups of mice, including controls. Swiss-Webster mice have an expected life span of only approximately a year, and the natural deaths of the control mice severely limited the statistical power of the study. Gross and microscopical examination of all major tissues and organs at necropsy revealed no significant neoplastic or non-neoplastic alterations that could be attributed to treatment with 4-nitrophenol. Hematological and clinical chemistry end points were not monitored. This study recently (July 9, 1991) underwent review by a peer review panel; the panel concluded that under the conditions of the study there was no evidence of carcinogenic activity in male or female Swiss-Webster mice.

NTP (1991) also conducted genotoxicity studies with 4-nitrophenol. 4-Nitrophenol was not mutagenic in S. typhimurium strains TA98, TAlOO, TA1535, and TA1537 in concentrations of up to 3,333 µg/plate with or without metabolic activation. 4-Nitrophenol did not induce sister chromatid exchange in Chinese hamster ovary cells in the absence or presence of metabolic activation at concentration levels of up to 500 µg/mL, but induced chromosomal aberrations in Chinese hamster ovary cells, in the presence of S-9, at concentrations that delayed cell cycle (1,500 µg/mL). No evidence of mutagenicity was found in germ cells of male Drosophila melanogaster administered 4-nitrophenol in feed (7,500 ppm) or by injection (1,500 ppm). The peer review comments regarding these studies were not available at the time of this writing.

The NIEHS has sponsored a carcinogenicity study with 4-nitrophenol to be conducted FY 1990 by Litton Bionetics, Inc. (NTP 1990). In addition, NIEHS sponsored a mutagenesis/genetic toxicity study with 4-nitrophenol to be completed FY 1990; the performing organization was not specified (NTP 1990). An acute/chronic toxicity study on 4-nitrophenol sponsored by the FDA was to be completed FY 1990 (NTP 1990). 4-Nitrophenol has been selected for a pharmacokinetics/metabolism study by EPA (NTP 1990); this research is to be conducted at the Health Effects Research Laboratory.

No on-going studies were identified regarding 2-nitrophenol.