

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 Otto Fuel II and its components, 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 Otto Fuel II and its components based on toxicological studies and epidemiological investigations.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

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 (14 days or less), 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

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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 and extrapolation of data from laboratory animals to humans.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), 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 effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

Otto Fuel II is a torpedo propellant that is composed of a mixture of approximately 75% propylene glycol dinitrate, 2% 2-nitrodiphenylamine, and 23% dibutyl sebacate. The studies described in this section include both those concerning exposures to the mixture, Otto Fuel II, as well as those concerning exposures to the individual components of this mixture.

Propylene glycol dinitrate is the only component of Otto Fuel II with significant volatility. However, inhalation exposures to the other components of Otto Fuel II are considered in this section because mists of Otto Fuel II may be generated, and inhalation of aerosol particles in the mists is possible.

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Occupational exposure to Otto Fuel II may result in simultaneous inhalation and dermal exposures. Thus, many of the effects reported in occupational studies in this section may be due, in part, to dermal exposure to Otto Fuel II.

2.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to Otto Fuel II or its individual components.

No studies were located regarding death in animals after inhalation exposure to either Otto Fuel II or two of its components, 2-nitrodiphenylamine, and dibutyl sebacate. However, information was available regarding death in animals following inhalation exposure to propylene glycol dinitrate. These studies indicate that differences in species sensitivity to the lethal effects of propylene glycol dinitrate may exist.

For example, exposure to concentrations of propylene glycol dinitrate as high as 200 ppm for 4 hours has been tolerated in rats without any toxic signs, and continuous (24 hours/day) exposure of rats, guinea pigs, and dogs to 35 ppm for 90 days produced no treatment-related deaths in any of these species (Jones et al. 1972). However, continuous exposure of a rabbit to 35 ppm of propylene glycol dinitrate resulted in the death of the rabbit on the 4th day (Jones et al. 1972). The rabbit appeared to be cyanotic 6 hours prior to death. One out of 9 squirrel monkeys exposed continuously to 35 ppm died on the 31st day of exposure, and a squirrel monkey exposed to 61 ppm of propylene glycol dinitrate for 23 hours/day died on the third day of exposure (Jones et al. 1972).

In longer-term studies, intermittent (6 hours/day, 5 days/week) exposure of rats and mice to 35 ppm of propylene glycol dinitrate for 1 year resulted in no increase in deaths over the death rates of controls in either species during the exposure period or in the 1-year postexposure observation period (Air Force 1985a). Also, a 14-month exposure of dogs to 0.2 ppm propylene glycol dinitrate for 6 hours/day, 5 days/week, caused no treatment-related deaths (Air Force 1985a). Test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

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2.2.1.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for each study for each end point for propylene glycol dinitrate are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to 2-nitrodiphenylamine or dibutyl sebacate. However, information was available regarding respiratory end points following inhalation exposure to Otto Fuel II and propylene glycol dinitrate.

Approximately 6% of a group of 87 torpedo maintenance workers with 1-132 months (average 47 months) of occupational exposure to Otto Fuel II reported experiencing dyspnea during past exposures (Horvath et al. 1981). However, it is unknown whether the incidence of this effect was increased relative to controls. Airway irritation was reported by incinerator workers with between 17 months and 3 years of experience (ATSDR 1990). In addition, one individual living near the incinerator was reported to have had bronchiolar obstruction; possibly related to inhaling incinerator fumes. However, only approximately 10% of the material incinerated was Otto Fuel II; other solvents and corrosive materials were also incinerated. Thus, the irritation and bronchiolar damage described in the report by ATSDR (1990) cannot be entirely attributed to Otto Fuel II exposure with certainty.

Nasal congestion was reported by approximately 31% of the torpedo maintenance workers in the study by Horvath et al. (1981). Although respiratory tissues were affected, this symptom is most likely due to a direct vasodilatory effect of the propylene glycol dinitrate on blood vessels of the nasal mucosa and not to an effect on respiratory function. Therefore, nasal congestion is also discussed below as a symptom of a cardiovascular response.

Volunteers exposed, in a single session lasting between 1 and 8 hours, to propylene glycol dinitrate atmospheres generated by volatilizing Otto Fuel II experienced no effects on respiratory function as measured using computerized spirometry (Stewart et al. 1974). Exposure levels in the study by Stewart et al. (1974) were measured by monitoring atmospheric concentrations of propylene glycol dinitrate. Exposure to concentrations of propylene glycol dinitrate in this study as high as 1.5 ppm for a single 3.2-hour period or 0.2 ppm for 7.5-8 hours/day for 5 days caused no measurable decrease in the respiratory function parameters measured.

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
ACUTE EXPOSURE							
Systemic							
1	Human	5 d 7.5-8hr/d	Resp Cardio Hemato Ocular	0.2 0.2 0.2 0.2			Stewart et al. 1974
2	Human	1-8 hr	Resp Cardio Hemato Ocular	1.5 0.35 1.5 0.5	0.5 (12 mm Hg increase diastolic blood pressure) 1.5 (definite eye irritation in 8/8)		Stewart et al. 1974
3	Rat (NS)	4 hr	Hemato		200 (23.5% methemoglobin)		Jones et al. 1972
4	Dog (Beagle)	2 wk 5d/wk 6hr/d	Hemato		0.2 (decreased hematocrit and hemoglobin)		Air Force 1985a
Neurological							
5	Human	once 30-60 min (occup)			≤0.22 (decreased performance in oculomotor and ataxia tests)		Horvath et al. 1981
6	Human	5 d 7.5- 8 hr/d			0.2 (altered visual evoked response; headache)		Stewart et al. 1974
7	Human	1-8 hr		0.1 ^b	0.2 (altered visual evoked response; headache)	0.5 (ataxia)	Stewart et al. 1974

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
INTERMEDIATE EXPOSURE							
Systemic							
8	Monkey (Squirrel)	90 d 24hr/d	Resp	35 M			Jones et al. 1972
			Cardio	35 M			
			Hemato	10 M		16 M (hemolysis)	
			Hepatic	10 M	16 M (hemosiderin deposits in the liver, fatty changes)		
			Renal	10 M		16 M (hemosiderin deposits in the kidneys, elevated BUN)	
			Bd Wt	35 M			
9	Rat (Fischer 344)	2-51 wk 5d/wk 6hr/d	Hemato			35 (increased methemoglobin; decreased red blood cells)	Air Force 1985a
			Bd Wt	35			
10	Rat (Sprague-Dawley)	90 d 24hr/d	Resp	35			Jones et al. 1972
			Cardio	35		10 (hemolysis)	
			Hemato			35 F (focal necrosis)	
			Hepatic		10 (fatty change in liver)		
			Renal		10 (hemosiderin deposits in the kidneys)	35 F (tubular necrosis)	
			Bd Wt	35			
11	Dog (Beagle)	2-51 wk 5d/wk 6hr/d	Hemato		0.2 ^c (decreased hematocrit, hemoglobin, red blood cells, reticulocytes; increased methemoglobin)		Air Force 1985a

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
12	Dog (Beagle)	90 d 24hr/d	Resp	35 M			Jones et al. 1972
			Cardio	35 M			
			Hemato			10 M (hemolysis)	
			Hepatic		10 M (hemosiderin deposits in the liver)		
			Renal		10 M (hemosiderin deposits in the kidneys)		
			Endocr	35			
			Bd Wt	35			
13	Gn pig (Hartley)	90 d 24hr/d	Resp	10	16 (foci of pulmonary hemorrhage)		Jones et al. 1972
			Cardio	35			
			Hemato		35 (elevated methemoglobin)		
			Hepatic		10 (fatty change in the liver)		
			Renal	35			
			Bd Wt	35			
Immuno/Lymphor							
14	Monkey (Squirrel)	90 d 24hr/d		16 M	35M (hemosiderin deposits in spleen)		Jones et al. 1972
15	Rat (Sprague- Dawley)	90 d 24hr/d		35			Jones et al. 1972
16	Dog (Beagle)	90 d 24hr/d		16 M	35M (hemosiderin deposits in spleen)		Jones et al. 1972
17	Gn pig (Hartley)	90 d 24hr/d		35			Jones et al. 1972

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
Neurological							
18	Monkey (Squirrel)	90 d 24hr/d			39	(pupillary dilation)	Jones et al. 1972
19	Monkey (Squirrel)	90 d 24hr/d		35 M			Jones et al. 1972
20	Dog (Beagle)	90 d 24hr/d		35 M			Jones et al. 1972
CHRONIC EXPOSURE							
Systemic							
21	Rat (Fischer 344)	1 yr 5d/wk 6hr/d	Resp		0.2 M	(mild degeneration of nasal epithelium)	Air Force 1985a
			Cardio	35			
			Gastro	35			
			Hemato		35	(increased methemoglobin)	
			Musc/skel	0.2			
			Hepatic	35			
			Renal	35			
			Endocr	35			
			Dermal	35			
			Bd Wt	35			

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
22	Mouse (C57BL/6)	1 yr 5d/wk 6hr/d	Resp	35			Air Force 1985a
			Cardio	35			
			Gastro	35			
			Musc/skel	35			
			Hepatic	35			
			Renal	35			
			Endocr	35			
			Dermal	35			
			Bd Wt	35			
23	Dog (Beagle)	14 mo 5d/wk 6hr/d	Resp	0.2			Air Force 1985a
			Cardio	0.2			
			Gastro	0.2			
			Hemato		0.2 ^d	(decreased hematocrit, hemoglobin, red blood cells, reticulocytes; increased methemoglobin)	
			Musc/skel	0.2			
			Hepatic	0.2			
			Renal	0.2			
			Endocr	0.2			
			Dermal	0.2			
			Bd Wt	0.2			
24	Rat (Fischer 344)	1 yr 5d/wk 6hr/d	Immuno/Lymphor	35			Air Force 1985a

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
25	Mouse (C57BL/6)	1 yr 5d/wk 6hr/d		35			Air Force 1985a
26	Dog (Beagle)	14 mo 5d/wk 6hr/d		0.2			Air Force 1985a
Neurological							
27	Rat (Fischer 344)	1 yr 5d/wk 6hr/d		35			Air Force 1985a
28	Mouse (C57BL/6)	1 yr 5d/wk 6hr/d		35			Air Force 1985a
29	Dog (Beagle)	14 mo 5d/wk 6hr/d		0.2			Air Force 1985a
Reproductive							
30	Rat (Fischer 344)	1 yr 5d/wk 6hr/d		35			Air Force 1985a
31	Mouse (C57BL/6)	1 yr 5d/wk 6hr/d		35			Air Force 1985a

TABLE 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate - Inhalation (continued)

Key to figure ^a	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
32	Dog (Beagle)	14 mo 5d/wk 6hr/d		0.2			Air Force 1985a

^aThe number corresponds to entries in Figure 2-1.

^bUsed to derive an acute inhalation Minimal Risk Level (MRL) of 0.003 ppm; dose adjusted from intermittent to continuous dosing (0.1 ppm x 8 hr/24 hr = 0.03 ppm); adjusted dose divided by an uncertainty factor of 10 (for human variability).

^cUsed to derive an intermediate inhalation MRL of 0.00004 ppm; dose adjusted from intermittent to continuous dosing (0.2 ppm x 5 d/7 d x 6 hr/24 hr = 0.036 ppm); adjusted dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

^dUsed to derive a chronic inhalation MRL of 0.00004 ppm; dose adjusted from intermittent to continuous dosing (0.2 ppm x 5 d/7 d x 6 hr/24 hr = 0.036 ppm); adjusted dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno./Lympho = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate – Inhalation

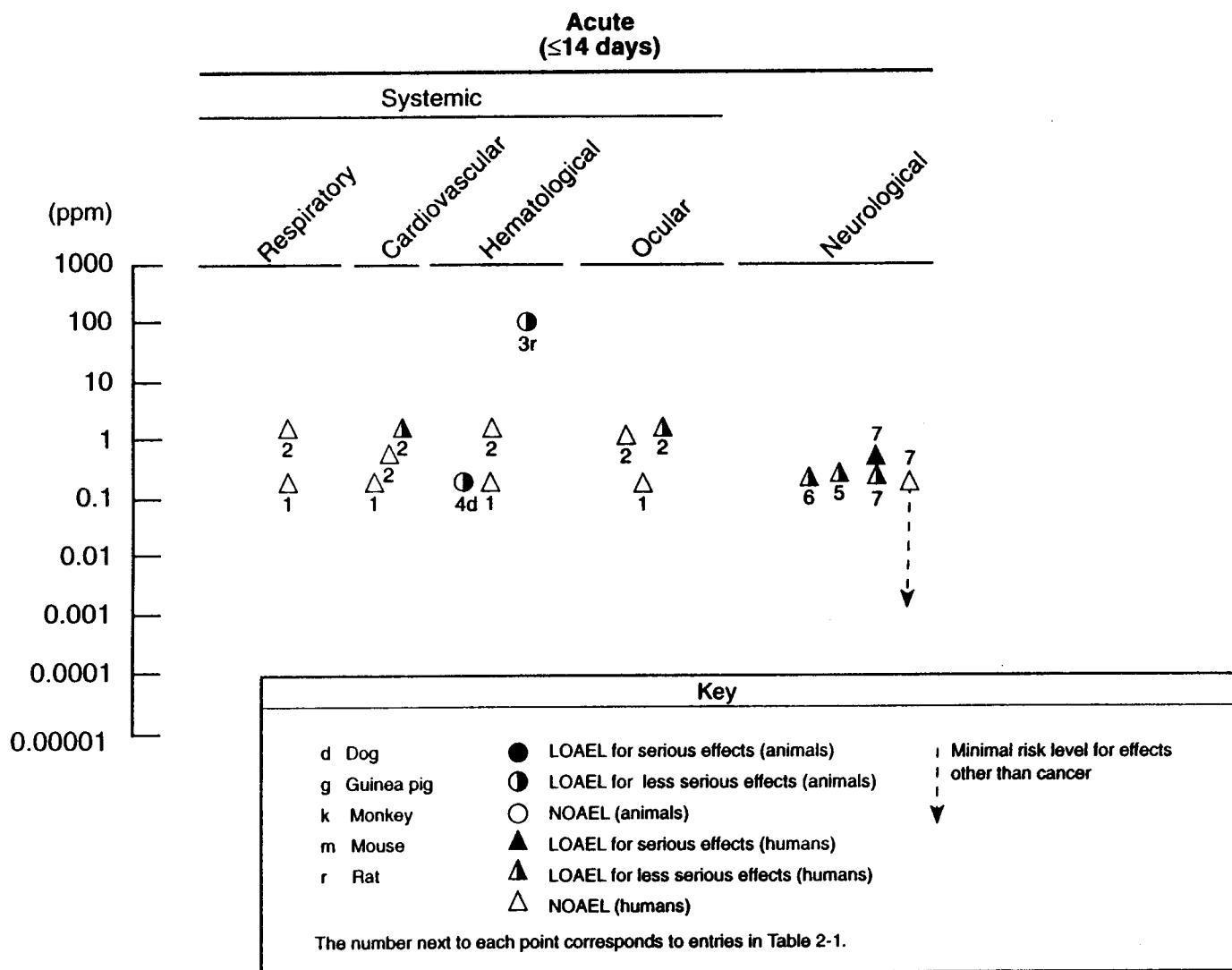


Figure 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate – Inhalation (continued)

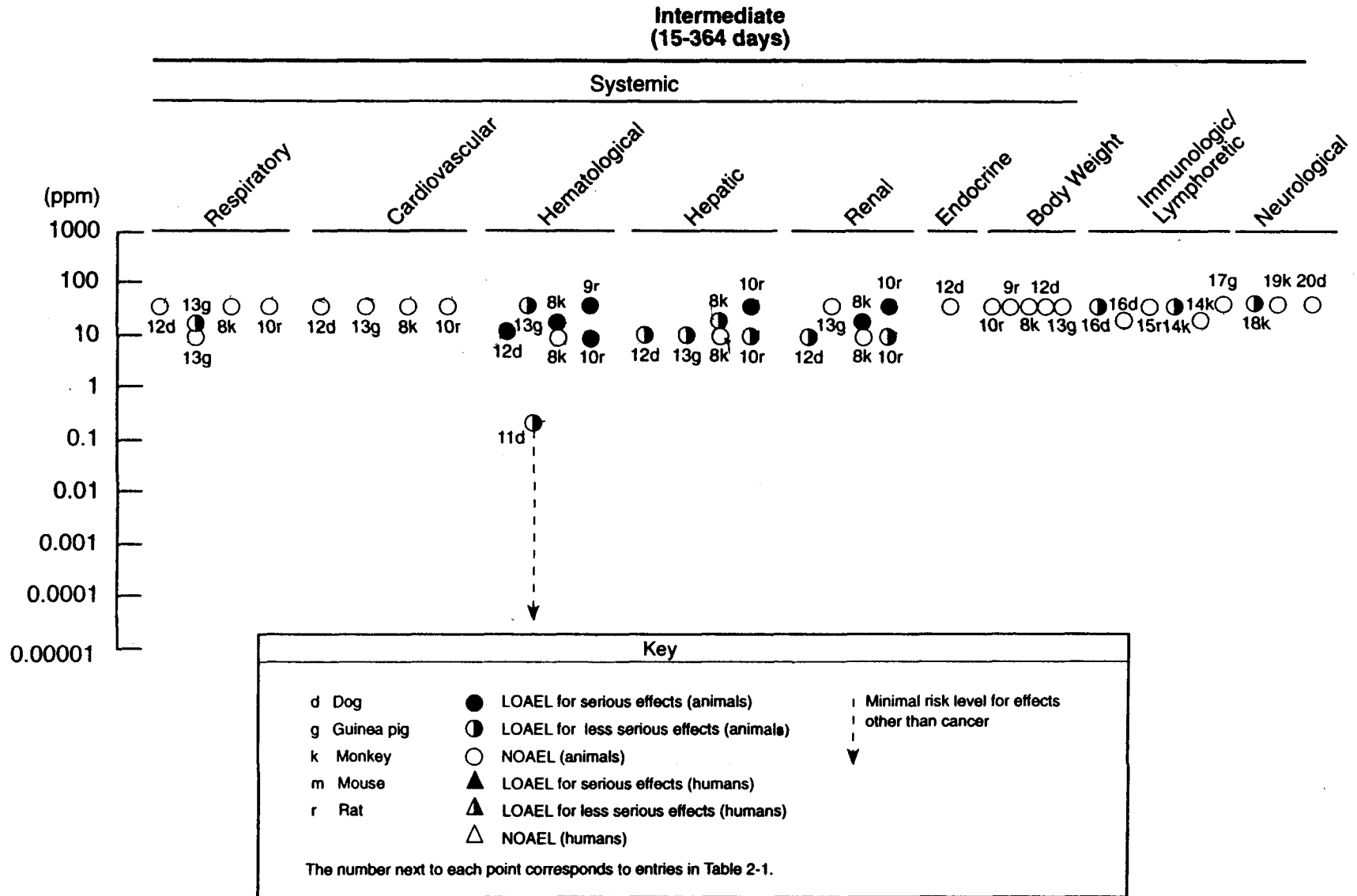
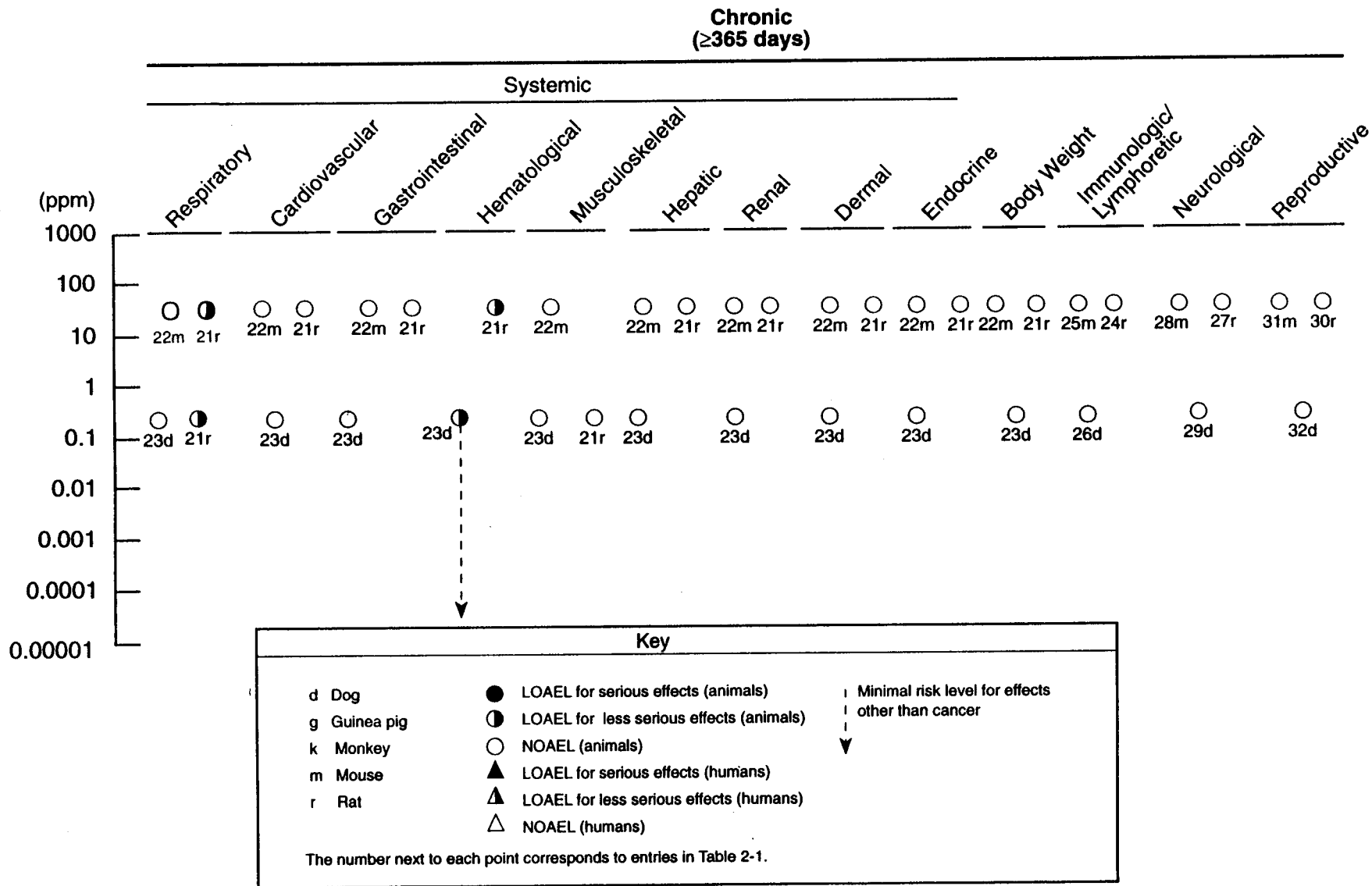


Figure 2-1. Levels of Significant Exposure to Propylene Glycol Dinitrate – Inhalation (continued)



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No studies were located regarding respiratory effects in animals after inhalation exposure to Otto Fuel II, 2-nitrodiphenylamine, or dibutyl sebacate. However, studies were located that examined respiratory end points in animals following inhalation exposure to propylene glycol dinitrate.

In general, inhalation exposure of animals to propylene glycol dinitrate resulted in no gross or histologically apparent adverse effects on the tissues of the respiratory system. Studies examining the effects of a 90-day, 24-hour/day exposure of rats, monkeys, and dogs to 35 ppm propylene glycol dinitrate showed no treatment-related gross or histopathological alterations in the lungs (Jones et al. 1972). In addition, a 1-year exposure of mice to 35 ppm propylene glycol dinitrate and a 14-month exposure of dogs to 0.2 ppm propylene glycol dinitrate for 6 hours/day, 5 days/week, produced no gross or histopathological changes in the tissues of the nasal cavity, larynx, trachea, bronchi, or lungs (Air Force 1985a). In this Air Force study (1985a), the atmosphere containing propylene glycol dinitrate was generated by volatilizing Otto Fuel II. The study is limited, however, in that the controls were not sham exposed. In addition, the control mice developed intercurrent ulcerative dermatitis, thereby confounding the conclusions that can be drawn.

Adverse respiratory effects were, however, observed in two studies. In the first study, exposure of guinea pigs to 16 ppm propylene glycol dinitrate for 24 hours/day for 90 days resulted in foci of pulmonary hemorrhage in both males and females (Jones et al. 1972). In the other study, exposure of rats of both sexes to propylene glycol dinitrate for 6 hours/day, 5 days/week, for 1 year, resulted in mild degeneration of the nasal epithelium at concentrations as low as 0.2 ppm and pulmonary inflammation only in females at 35 ppm (Air Force 1985a). After a 1-year recovery period, the effects on the nasal epithelium and lungs were no longer significantly increased. As noted above, the propylene glycol dinitrate atmospheres in the Air Force study were generated by volatilizing Otto Fuel II. Also, controls were not sham exposed in this study; thus, it is difficult to determine whether the effects observed on the nasal epithelium and lungs were related specifically to propylene glycol dinitrate exposure.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans following inhalation exposure to 2-nitrodiphenylamine alone or dibutyl sebacate alone. However, information was available regarding cardiovascular end points following inhalation exposure to Otto Fuel II or propylene glycol dinitrate.

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Symptoms identified with nitrate-induced vasodilation or compensatory vasoconstriction have been observed in studies examining human exposure to Otto Fuel II and its major component, propylene glycol dinitrate. For example, headaches of presumed vascular origin were experienced either frequently or occasionally by 65% of 87 torpedo maintenance workers with 1-132 months (average of 47 months) of exposure to Otto Fuel II (Horvath et al. 1981). However, it was not reported whether the incidence of this effect was greater than in controls. Also, workers at an incinerator where Otto Fuel II was burned reported that they were able to identify exposures to Otto Fuel II by the characteristic vasodilatory headache (ATSDR 1990).

Experimental exposure of volunteers to atmospheres of propylene glycol dinitrate generated by volatilizing this chemical from Otto Fuel II, resulted in headaches of presumed vascular origin (Stewart et al. 1974). The headaches began as mild frontal headaches and became progressively worse and throbbing in nature. Headaches were reported by some subjects exposed to concentrations of propylene glycol dinitrate as low as 0.2 ppm for up to 8 hours. With repeated exposures, the severity and frequency of headaches was observed to decrease. By analogy with the effects of other aliphatic nitrates, the headaches are most likely the result of vasodilation of the meningeal blood vessels (Nickerson 1975).

Nasal congestion was also reported by 31% of the torpedo maintenance workers interviewed by Horvath et al. (1981) as an occasional or frequent symptom associated with Otto Fuel II exposure. This effect was considered to be cardiovascular in origin because it was most likely the result of vasodilation of blood vessels in the nasal mucosa and sinuses.

Lowered blood pressures were observed in workers at an incineration facility at which Otto Fuel II comprised approximately 10% of the waste burned (ATSDR 1990). However, this effect cannot be directly attributed to Otto Fuel II exposure because of the number of other materials incinerated at the facility. An increase in diastolic pressure was observed during the last 2-3 hours of exposure in volunteers exposed to 0.5 ppm propylene glycol dinitrate for 8 hours (Stewart et al. 1974) possibly indicating compensatory vasoconstriction.

Based on the observation that nitrated esters cause a compensatory vasospasm that may produce coronary insufficiency upon withdrawal from exposure (Carmichael and Lieben 1963; Morton 1977), a study was conducted to examine the incidence of coronary symptoms among 1,352 Navy torpedoman's

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mates with potential exposure to Otto Fuel II (Forman et al. 1987). This study demonstrated a significantly greater incidence of hospitalizations for myocardial infarctions or angina pectoris in the exposed torpedoman's mates than in 14,336 unexposed torpedoman's mates or 29,129 fire control technicians. Palpitations and chest pain were also reported by the workers in the study by Horvath et al. (1981). Of the 87 exposed workers that were interviewed, 10% reported experiencing palpitations and 4% reported experiencing chest pain. However, it was not reported whether the incidence of these symptoms was greater than in the controls. Chest pain and tightness were also reported by several workers at an incinerator where Otto Fuel II was burned (ATSDR 1990). One of these workers was diagnosed with nitrate withdrawal angina when cardiac catheterization failed to reveal any other cause. However, causative agents other than Otto Fuel II cannot be eliminated because of the large number of other hazardous wastes also burned at the facility.

No changes were observed in the electrical activity or mechanical performance of the hearts of 9 subjects exposed to up to 0.5 ppm propylene glycol dinitrate for 8 hours, 1.5 ppm propylene glycol dinitrate for 3.2 hours, or 0.2 ppm propylene glycol dinitrate for 5 days for 7.5-8 hours/day (Stewart et al. 1974). Tests were conducted during exposure, and baseline studies were repeated 16 hours following each exposure.

No studies were located regarding cardiovascular effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, limited information was located regarding cardiovascular effects in animals following inhalation exposure to propylene glycol dinitrate.

Relative to a pre-exposure period, anesthetized rats exposed nose-only to up to 94 ppm propylene glycol dinitrate vapors for 30-45 minutes exhibited no changes in mean systolic pressure during exposure (Godin et al. 1993). Exposure of monkeys to 74-103 ppm of propylene glycol dinitrate for 6 hours resulted in pallor and cold extremities of the monkeys during exposure (Jones et al. 1972), suggesting hypotension and compensatory vasoconstriction. The monkeys recovered within 30-45 minutes after termination of the exposure. No other studies reported effects associated with changes in vascular tone. However, two studies found no gross or microscopically apparent damage to the heart following inhalation exposure to propylene glycol dinitrate. In one study, no gross or histopathologic changes in the heart were observed following exposure of rats, guinea pigs, dogs, or monkeys to 35 ppm propylene glycol dinitrate for 24 hours/day, for 90 days (Jones et al. 1972). In

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the other study, no gross or histopathologic changes in the heart were observed during routine examination of tissues from rats or mice exposed to 35 ppm propylene glycol dinitrate for 6 hours/day, 5 days/week, for 1 year, or from dogs exposed to 0.2 ppm propylene glycol dinitrate for 6 hours/day, 5 days/week, for 14 months (Air Force 1985a). The test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to Otto Fuel II or its components.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine or dibutyl sebacate. However, extremely limited information was located regarding potential gastrointestinal effects resulting from inhalation exposure of animals to propylene glycol dinitrate.

Routine gross and histopathological examination of the esophagus, stomach, duodenum, ileum, colon, and anus of rats and mice exposed to 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week, and then observed for an additional year post-exposure revealed no treatment-related effects (Air Force 1985a). Similarly, no treatment-related gross or microscopic changes were observed in this study in dogs exposed to 0.2 ppm propylene glycol dinitrate for 14 months, 6 hours/day, 5 days/week.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, methemoglobin levels were examined in one study in which volunteers were exposed to concentrations of 1.5 ppm propylene glycol dinitrate for 3.2 hours or 0.2 ppm for 7.5-8 hours/day for 5 days (Stewart et al. 1974). Inhalation exposure at these levels was insufficient to elevate methemoglobin levels above control values. Test atmospheres in this study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

No studies were located regarding hematological effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, hematological end points were assessed in several studies following inhalation exposure of animals to propylene glycol dinitrate.

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Elevated methemoglobin levels were observed in a number of acute-, intermediate-, and chronic-duration exposure studies in which animals were exposed to propylene glycol dinitrate. Methemoglobin levels of 23.5% were measured in rats immediately following a 4-hour exposure to 200 ppm propylene glycol dinitrate (Jones et al. 1972). Cyanosis and elevated methemoglobin levels (18.2%) were observed in a rabbit exposed to 35 ppm propylene glycol dinitrate for 23 hours/day for 4 days (Jones et al. 1972). This animal died on the 4th day of exposure. At the time of death, the methemoglobin level was 32.8%. Similarly, the methemoglobin level in a squirrel monkey that died following exposure to 61 ppm propylene glycol dinitrate for 23 hours/day for 3 days was 40.2% at the time of death (Jones et al. 1972).

Continuous (24 hours/day) exposure of rats, guinea pigs, dogs, and monkeys to propylene glycol dinitrate for 90 days resulted in elevated methemoglobin levels during exposure and histopathologic evidence of hemolysis in all four species (Jones et al. 1972). At concentrations as low as 10 ppm, hemosiderin deposits (indicating phagocytosis of oxidized hemoglobin released from hemolyzed red cells) were observed in kidneys and livers from dogs and in kidneys from some rats. At 16 ppm, hemosiderin deposits were observed in the liver of dogs and monkeys; at 35 ppm, in addition to the liver and kidneys, heavy hemosiderin deposits were observed in the spleens of these animals. At 35 ppm, all four species exhibited elevated methemoglobin levels. Postexposure values for leukocytes, hemoglobin, and hematocrit were not significantly altered in rats, guinea pigs, or monkeys, but dogs exposed to 35 ppm experienced an average 63% decrease in hemoglobin and a 37% decrease in hematocrit (Jones et al. 1972).

Chronic intermittent (6 hours/day, 5 days/week) exposure of dogs to 0.2 ppm propylene glycol dinitrate for 14 months resulted in a small but statistically significant increase in methemoglobin levels (1-3% above normal levels) (Air Force 1985a). Exposure at this level also caused anemia in the dogs. At the first 2-week blood test, a decreased hematocrit and hemoglobin content was observed, and at 4 weeks, a significant decrease in the number of red blood cells and reticulocytes was also observed. These decreases were maintained throughout the duration of the study. Despite the indication that hematopoiesis was affected (low reticulocyte count in the presence of reduced red cell count), samples of bone marrow taken at necropsy showed no effect on the blood forming cells and no effect was observed on the spleen. Additional tests performed to determine the nature of the anemia revealed neither excessive hemoglobin denaturation nor increase in the fragility of the erythrocyte membrane. No Heinz bodies (precipitates of denatured hemoglobin within the red blood cell) were observed, and

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the erythrocytes demonstrated only a Slight increase in osmotic fragility at the lowest osmotic strengths tested. Rats exposed to 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week, also demonstrated a small but statistically significant increase in methemoglobin levels (1-2% above normal) during exposure, but no consistent effects on the hematocrit, hemoglobin, or red blood cell content of the blood were observed (Air Force 1985a), indicating the increased sensitivity of the dog relative to the rat. The test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, a “seronegative arthritis” was diagnosed in several workers from an incinerator at which 10% of the material burned was Otto Fuel II (ATSDR 1990). Because of the large number of other wastes handled at the facility, the arthritis cannot be ascribed to Otto Fuel II exposure with any certainty.

No studies were located regarding musculoskeletal effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, effects on the skeletal system were observed in one animal study following inhalation exposure to propylene glycol dinitrate.

An increased incidence of osteosclerosis of cortical bone (sternbrae, vertebrae and/or femur) was observed in female rats 1 year after they had been exposed to 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week (Air Force 1985a). This effect was attributed by the study authors to altered estrogen levels in these animals, but no data on estrogen levels were presented to support this assertion. Routine gross and histopathologic examination of the thigh muscle and sternbrae, vertebrae, or femur of mice exposed to 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week, and then observed for an additional year, or of dogs exposed to 0.2 ppm propylene glycol dinitrate for 14 months, 6 hours/day, 5 days/week, revealed no abnormalities in these tissues (Air Force 1985a). The test atmospheres in this study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, transient

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elevations were noted in unspecified parameters assessing liver function in several incinerator workers at a facility at which approximately 10% of the waste handled was Otto Fuel II (ATSDR 1990). This effect cannot be definitively attributed to Otto Fuel II because the other wastes incinerated included a number of solvents.

No studies were located regarding hepatic effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, several animal studies provided information regarding hepatic end points following inhalation exposure to propylene glycol dinitrate.

Continuous (24 hours/day) exposure of rats, guinea pigs, dogs, and monkeys to propylene glycol dinitrate for 90 days has been shown to result in degenerative changes in the liver (Jones et al. 1972). Concentrations of propylene glycol dinitrate as low as 10 ppm in rats and guinea pigs, and 16 ppm in dogs and monkeys, have been shown to cause fatty changes in the livers of these animals. Changes observed at 35 ppm included vacuolar changes in the livers of guinea pigs, focal liver necrosis in female rats (male rats were unaffected), and vacuolar change, mononuclear, cell infiltrates, and focal necrosis in the livers of monkeys and dogs (Jones et al. 1972). In addition, bromosulfophthalein retention was increased in one of the two dogs exposed at 35 ppm, indicating depressed hepatic function. In dogs and monkeys, the degenerative changes were commonly associated with heavy deposition of hemosiderin in the liver. It is unclear whether these effects are causally related.

In contrast, routine gross and histopathological examination of livers from animals that were intermittently exposed to propylene glycol dinitrate for intermediate or chronic durations did not reveal any adverse effects. For example, gross and histopathological examination of livers of male rats exposed to 10 ppm propylene glycol dinitrate for 6 weeks, 7 hours/day, 5 days/week (Jones et al. 1972) or 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week (Air Force 1985a) showed no treatment-related effects. The 6-week study is limited in that no controls were used. Gross and histopathological examination of livers of mice exposed for 1 year, 6 hours/day, 5 days/week, to 35 ppm propylene glycol dinitrate or dogs exposed for 14 months, 6 hours/day, 5 days/week, to 0.2 ppm propylene glycol dinitrate revealed no adverse treatment-related effects (Air Force 1985a). The test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

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Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to Otto Fuel II or its components.

No studies were located regarding renal effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, several studies have examined the renal toxicity of propylene glycol dinitrate following inhalation exposure.

Continuous (24 hours/day) exposure of rats to 35 ppm of propylene glycol dinitrate for 90 days resulted in an increased incidence of acute tubular necrosis in female, but not male, rats (Jones et al. 1972). In addition, continuous exposure of monkeys to propylene glycol dinitrate at concentrations as low as 16 ppm for 90 days resulted in increased serum urea nitrogen and decreased serum alkaline phosphatase (indicating the possibility of renal dysfunction). However, no gross or histological evidence of degenerative changes in the kidneys was observed in monkeys exposed to concentrations as high as 35 ppm for 90 days (Jones et al. 1972). The renal toxicity observed in these cases may have been caused by hemoglobinuria resulting from massive hemolysis (Cotran et al. 1989). In support of this is the observation that continuous exposure of rats to concentrations as low as 10 ppm and monkeys to concentrations as low as 16 ppm of propylene glycol dinitrate for 90 days resulted in an increase in hemosiderin deposits in the proximal convoluted tubules of the kidneys (Jones et al. 1972). Heavy hemosiderin deposits were observed in the kidneys of monkeys continuously exposed to 35 ppm propylene glycol dinitrate for 90 days. These deposits were attributed to increased destruction of erythrocytes (see also Hematological Effects above). Arguing against the role of hemoglobinuria in the renal toxicity is the observation that dogs exposed to concentrations of propylene glycol dinitrate as low as 10 ppm also had increased hemosiderin deposits in the proximal convoluted tubules of the kidneys but no clinical or microscopic evidence of renal dysfunction at concentrations as high as 35 ppm (Jones et al. 1972). Thus, it is unclear whether the observed renal effects in female rats and monkeys were the result of hemoglobinuria or a direct toxic effect of propylene glycol dinitrate on the kidneys.

Other studies in which intermittent exposures were used have not detected renal toxicity. Routine gross and histopathological examination of kidneys from male rats that have been intermittently (7 hours/day, 5 days/week, for 6 weeks) exposed to 10 ppm of propylene glycol dinitrate by inhalation (Jones et al. 1972) or from rats or mice that have been intermittently (6 hours/day, 5 days/week, for 1 year) exposed by inhalation to 35 ppm propylene glycol dinitrate (Air Force 1985a) did not reveal

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any adverse effects. The 6-week study by Jones et al. (1972) is limited in that no controls and only male rats were used. The test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

Endocrine Effects. Limited information is available regarding endocrine effects after inhalation exposure to Otto Fuel II and its components. No gross or histopathologic alterations were observed in the adrenals of two male dogs continuously exposed to up to 35 ppm propylene glycol dinitrate for 90 days (Jones et al. 1972). In a chronic-duration study, no gross or microscopical alterations were observed in the thyroid, parathyroid, and pituitary glands from mice and rats exposed for a year to 35 ppm of propylene glycol dinitrate vapors 6 hours/day, 5 days/week, or in dogs exposed to 0.2 ppm using a similar protocol (Air Force 1985a).

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to propylene glycol dinitrate, 2-nitrodiphenylamine or dibutyl sebacate. Eczema, dermatitis with macropapular rashes, and sweating with no apparent cause were reported by incinerator workers at a facility at which Otto Fuel II made up approximately 10% of the waste material handled (ATSDR 1990). However, other materials processed at the incinerator could have been responsible for these effects.

No studies were located regarding dermal effects in animals after inhalation exposure to Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate. No adverse effects of inhalation exposure to propylene glycol dinitrate on the skin were reported in rats or mice exposed to 35 ppm for 1 year, 6 hours/day, 5 days/week, or in dogs exposed to 0.2 ppm for 14 months, 6 hours/day, 5 days/week (Air Force 1985a). The test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

Ocular Effects. No studies were located regarding ocular effects in humans after inhalation exposure to 2-nitrodiphenylamine and dibutyl sebacate. However, eye irritation has been reported by workers occupationally exposed to Otto Fuel II and volunteers experimentally exposed to propylene glycol dinitrate. Slight eye irritation was reported by one of twelve volunteers exposed to atmospheres containing 0.2 propylene glycol dinitrate in an exposure chamber for 8 hours (Stewart et al. 1974). An exposure level of 1.5 ppm propylene glycol dinitrate caused frank eye irritation in 8 out of 8 subjects after 40 minutes in the chamber (Stewart et al. 1974). However, the same investigators also reported

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that none of 9 volunteers exposed to 0.2 ppm propylene glycol dinitrate 8 hours/day for 5 days complained of eye irritation (Stewart et al. 1974). Eye irritation was also reported as an occasional or frequent symptom experienced by 26% of a group of 87 workers occupationally exposed to Otto Fuel II (Horvath et al. 1981). However, the incidence of this effect was not contrasted with the incidence in an unexposed population. Eye irritation was also a common complaint among incinerator workers at a facility at which Otto Fuel II made up approximately 10% of the waste materials handled (ATSDR 1990). However, other materials processed at the incinerator could have been responsible for these effects.

No studies were located regarding ocular effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, limited information on ocular end points was found in one study in which animals were exposed to propylene glycol dinitrate by inhalation.

Pupillary dilation was observed to increase from slight to moderate in monkeys exposed to atmospheres containing 35 ppm propylene glycol dinitrate for 24 hours/day for 90 days (Jones et al. 1972). It was not stated whether this effect was a direct effect on the eyes or whether the effect may have been neurologically mediated (see also Section 2.2.1.4).

Body Weight Effects. Body weight effects after inhalation exposure to Otto Fuel II or its components are limited to studies in animals exposed to propylene glycol dinitrate. No significant alterations in body weight gain were reported in rats, guinea pigs, dogs and monkeys exposed continuously to 35 ppm propylene glycol dinitrate vapors for 90 days (Jones et al. 1972). Similar results were reported in rats and mice exposed to 35 ppm and dogs exposed to 0.2 ppm intermittently for approximately one year (Air Force 1985a).

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after inhalation exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, an immunological end point was observed in a situation where workers were exposed to Otto Fuel II.

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A child of a worker at an incinerator at which Otto Fuel II was processed developed asthma (ATSDR 1990). The asthma was linked to materials handled at the incinerator because when the father of the child stopped bringing his work clothing home, the child's condition improved. However, Otto Fuel II comprised only approximately 10% of the wastes handled at the facility; thus, other materials may have triggered the reaction in the child. The possibility also exists that the asthma-like response may have been caused by tetryl particles activating nerve receptors which triggered vagal reflexes inducing bronchoconstriction.

No studies were located regarding immunological effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, limited information was located regarding immunological effects of propylene glycol dinitrate in animals after inhalation exposure.

No studies that directly examined immunological function were located. However, no gross or histopathologic alterations were observed in the spleens from rats and guinea pigs exposed continuously to up to 35 ppm propylene glycol dinitrate for 90 days (Jones et al. 1972). Dogs and monkeys exposed in the same fashion showed heavy iron deposits in their spleens at 35 ppm, but not at 16 ppm or less (Jones et al. 1972). Total and differential leukocyte counts and routine gross and histopathological examination of the mandibular and mesenteric lymph nodes, spleen and thymus were normal in rats and mice exposed to 35 ppm propylene glycol dinitrate for 6 hours/day, 5 days/week, for 1 year, and in dogs exposed to 0.2 ppm for 6 hours/day, 5 days/week, for 14 months (Air Force 1985a). Test atmospheres in the Air Force study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

The highest NOAEL values for immunologic effects of propylene glycol in rats, mice, guinea pigs, monkeys, and dogs in intermediate-duration and chronic-duration studies are recorded in Table 2-1 and plotted in Figure 2- 1.

2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to 2-nitrodiphenylamine or dibutyl sebacate. However, neurological effects were reported in studies

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examining occupational exposure to Otto Fuel II and experimental inhalation exposure to propylene glycol dinitrate.

Studies designed to assess the neurological effects of Otto Fuel II and its component, propylene glycol dinitrate, in humans have indicated that an alteration of central nervous system activity may result from occupational exposures. In one study, workers were given tests of balance and oculomotor performance before and after a torpedo maintenance procedure (Horvath et al. 1981). The maintenance procedures were approximately 30-60 minutes in duration, and propylene glycol dinitrate concentrations measured in the work area ranged from 0 to 0.22 ppm (88% of the concentrations measured were less than 0.1 ppm and 50% were less than 0.05 ppm). Although no statistically significant decrement in balance was observed, some tests of oculomotor performance were significantly altered. Mean saccade velocity was significantly decreased and mean saccade delay was significantly increased in the workers. Saccade accuracy and smooth pursuit were not significantly affected. A decrease in saccade velocity and an increase in saccade delay have been observed in cases of central nervous system depression. Consistent with this effect was the observation that volunteers exposed to various concentrations of propylene glycol dinitrate also exhibited central nervous system effects (Stewart et al. 1974). Exposure to 0.2 ppm propylene glycol dinitrate for 1-8 hours altered the visual evoked responses measured in the subjects. With repeated 7.5-8 hour exposures to 0.2 ppm, the change in the visual evoked response was observed to increase in magnitude indicating a cumulative effect. Exposure to 0.5 ppm for 8 hours resulted in nausea, dizziness, and more markedly altered visual evoked responses. At this concentration, the subjects had significantly altered ability to perform the modified Romberg and heel-to-toe tests. At the highest concentration tested, 1.5 ppm, subjects experienced coordination deficits and altered visual evoked responses although they were exposed for less than 4 hours. The effects on visual evoked responses and coordination were consistent with central nervous system depression. The central nervous system effects noted in these studies are consistent with the reports of dizziness and loss of balance reported by 13% and 1%, respectively, of workers occupationally exposed to Otto Fuel II (Horvath et al. 1981). However, it is unknown whether the incidence of the effects reported by exposed workers was significantly greater than in controls. No significant differences between the results of tests of oculomotor function or coordination and balance were observed when workers occupationally exposed to Otto Fuel II for 1-132 months (47 months average) were compared to clerical and technical personnel with no known exposure to Otto Fuel II (Horvath et al. 1981).

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An unspecified number of workers at an incinerator at which Otto Fuel II comprised approximately 10% of the waste materials processed, were diagnosed with a number of neurological disorders (ATSDR 1990). These included mood disorders, cerebellar dysfunction (ataxia, tremor, nystagmus), cognitive disorders, memory loss, and possible vestibular dysfunction. Magnetic resonance imaging showed that two of these workers had cerebellar and cortical atrophy. However, the effects observed cannot be attributed to Otto Fuel II with certainty because a large number of solvents and other wastes were also handled at the incinerator.

Headaches have frequently been reported both by workers occupationally exposed to Otto Fuel II and volunteers experimentally exposed to propylene glycol dinitrate (ATSDR 1990; Horvath et al. 1981 ; Sfewart et al. 1974). The headaches are most likely associated with meningeal blood vessel dilation (Nickerson 1975) (see also Cardiovascular Effects in Section 2.2.1.2).

No studies were located regarding neurological effects in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, several studies have examined neurological end points following inhalation exposure of animals to propylene glycol dinitrate.

Monkeys exposed to concentrations of propylene glycol dinitrate as low as 2 ppm for 4 hours have demonstrated altered visual evoked responses (Mattsson et al. 1981); however, studies in animals have provided only very limited gross behavioral evidence of neurological effects of propylene glycol dinitrate. For example, exposure of an unspecified number of monkeys to 74-103 ppm propylene glycol dinitrate for 6 hours resulted in semiconsciousness and clonic convulsions (Jones et al. 1972). Recovery from these effects was observed within 30-45 minutes after termination of exposure. Also, monkeys exposed to 39 ppm propylene glycol dinitrate for 24 hours/day, for 90 days, experienced an increase in pupillary dilation from slight to moderate (Jones et al. 1972). No effect on visual discrimination or visual acuity was observed. This study was limited by the small number of animals used.

Other tests have not demonstrated neurological effects of propylene glycol dinitrate in animals. Performance of monkeys in an operant avoidance behavioral test was not affected by inhalation exposure to propylene glycol dinitrate at concentrations as high as 33 ppm for 4 hours or by exposure to concentrations as high as 4.23 ppm for 23 hours/day in an increasing dose paradigm lasting

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125 days (Mattsson et al. 1981). This study is limited in that only two monkeys were used. No gross or histopathological effects have been observed in sections from brain and spinal cord or sciatic nerve in dogs and monkeys exposed to 35 ppm, 24 hours/day, for 90 days (Jones et al. 1972), in rats and mice exposed to 35 ppm, 6 hours/day, 5 days/week, for 1 year, and in dogs exposed to 0.2 ppm, 6 hours/day, 5 days/week, for 14 months (Air Force 1985a).

The highest NOAEL values and all reliable LOAEL values for neurological effects of propylene glycol dinitrate in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to the individual components of Otto Fuel II. However, a study compared the incidence of spontaneous and induced abortions in female Navy enlisted personnel exposed to Otto Fuel II with that in two groups of enlisted female workers not exposed to Otto Fuel II and found no increase (Forman 1988). The number of female workers exposed to Otto Fuel II in this study was extremely small, limiting the sensitivity of the comparison.

No studies were located that examined the effects of Otto Fuel II or its individual components on reproductive performance in animals after inhalation exposure. Although no information was located regarding reproductive performance, limited data were available regarding effects of propylene glycol dinitrate on the gross and microscopic structure of reproductive organs. Routine gross and histopathological examination of the seminal vesicles, prostate, testes, ovaries, uterus, and mammary glands of mice and rats exposed to 35 ppm propylene glycol dinitrate for 1 year, 6 hours/day, 5 days/week, and dogs exposed to 0.2 ppm for 14 months, 6 hours/day, 5 days/week, revealed no treatment-related effects (Air Force 1985a). Test atmospheres in this study were generated by volatilizing propylene glycol dinitrate from Otto Fuel II.

The highest NOAEL values for reproductive effects in each species following chronic-duration inhalation exposure to propylene glycol dinitrate are recorded in Table 2- 1 and plotted in Figure 2- 1.

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2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, extremely limited information regarding developmental effects in children of workers occupationally exposed to Otto Fuel II was available.

A child with multiple birth defects was born to the wife of a worker at an incineration facility at which Otto Fuel II comprised approximately 10% of the waste processed (ATSDR 1990). The defects included club foot, imperforate anus, renal anomalies and persistence of the cloaca. Chromosomal studies on the child were normal. However, the birth defects cannot be definitively ascribed to Otto Fuel II exposure because other possible causes for the defects existed (i.e., exposure to other developmental toxicants) and the incidence of similar defects in a control population were not adequately characterized.

No studies were located regarding developmental effects in animals after inhalation exposure to Otto Fuel II or its components.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to Otto Fuel II or its components.

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans after inhalation exposure to Otto Fuel II or any of its individual components.

No studies were located regarding cancer in animals after inhalation exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate.

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Extremely limited information was located regarding cancer in animals after inhalation exposure to propylene glycol dinitrate. One osteoma and one osteosarcoma were observed in rats 1 year following exposure to 0.2 ppm of propylene glycol dinitrate for 1 year, 5 days/week, 6 hours/day; 2 osteosarcomas were observed in rats 1 year following exposure at the 35ppm level for the same duration (Air Force 1985a). These tumors are rare and could be indicative of a tumorigenic potential of propylene glycol dinitrate. However, the study authors concluded that the lack of a dose response given the large differences in doses tested suggested that the tumors were not exposure related. Mice exposed to 35 ppm of propylene glycol dinitrate for 6 hours/day, 5 days/week, for 1 year, and then observed for 1 year postexposure, had an increased incidence of lymphoid hyperplasia (considered to be indicative of early lymphoma, a common lesion in older mice) (Air Force 1985a). However, the study authors also concluded that this lesion was incidental and not exposure related. Both of these studies are limited in that they were less than lifetime studies (18 months in the mouse and 2 years in the rat), the controls were not sham exposed, and a chronic ulcerative dermatitis was observed in the control mice, confounding the analysis of the results of the study.

2.2.2 Oral Exposure

With the exception of acute oral LD₅₀ data, virtually all of the information on the effects of Otto Fuel II or its components after oral exposure comes from studies examining the oral toxicity of dibutyl sebacate. These studies show that dibutyl sebacate is a relatively nontoxic component of Otto Fuel II. The only toxicity observed with dibutyl sebacate is a slight depression of body weight of offspring from parental animals that had consumed large amounts of dibutyl sebacate prior to conception (Smith 1953).

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to Otto Fuel II or its components.

The only study that was located regarding death in animals after oral exposure to Otto Fuel II was an LD₅₀ determination in rats; however, very few experimental details were presented (Navy 1982b). This study reported an LD₅₀ value of 2,000 mg/kg.

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A number of studies have examined the acute oral toxicity of propylene glycol dinitrate in rats. Oral LD₅₀ values ranging from 250 to 1,190 mg/kg have been determined (Andersen and Mehl 1979; Clark and Litchfield 1969; Jones et al. 1972). The reason for the wide range of values obtained is not clear but may have been due to differences in the strain, sex, age, or weight of the rats tested. Different rat strains were used in all three studies: Sprague Dawley (Andersen and Mehl 1979) Alderley Park strain (Clark and Litchfield 1969), and Long Evans (Jones et al. 1972). Also, Andersen and Mehl (1979) and Jones et al. (1972) used only male rats; whereas, Clark and Litchfield (1969) used only females. All three studies described similar symptoms before death including prostration and anoxia. In addition, mild convulsions were observed in the rats prior to death in the study by Jones et al. (1972).

Dibutyl sebacate has very low toxicity and only very large oral doses were observed to result in death. All rats given a single oral dose of 32,000 mg/kg died; whereas, all rats given 16,000 mg/kg survived (Smith 1953). No gross or histopathologic changes indicating the cause of death were observed in the rats at necropsy. However, extremely large volumes of test material were administered (approximately 2.4 mL at the highest dose tested) to 60-75gram rats; thus, it is possible that death may have resulted from aspiration of the test material. This is supported by the observation of hemorrhaging and edema in the lungs of the rats and mice receiving doses greater than or equal to 10-15 g/kg in the oral LD₅₀ study by Komarova (1976, 1979). Oral LD₅₀ values for dibutyl sebacate in rats and mice in this study were reported to be 17,200 mg/kg/day and 18,100 mg/kg/day, respectively (Komarova 1976, 1979).

The only information located regarding death following ingestion of 2-nitrodiphenylamine indicated that the oral toxicity of 2-nitrodiphenylamine was also very low. The oral LD₅₀ value for rats was 6,150 mg/kg. (This information was obtained from a Material Safety Data Sheet on 2-nitrodiphenylamine [American Cyanamid 1982]). Because the actual study was unavailable for review, this value could not be verified.)

All reliable LOAEL values for death in rats following acute-duration exposure are recorded for propylene glycol dinitrate in Table 2-2 and for dibutyl sebacate in Table 2-3 and plotted for propylene glycol dinitrate in Figure 2-2 and for dibutyl sebacate in Figure 2-3.

TABLE 2-2. Levels of Significant Exposure to Propylene Glycol Dinitrate - Oral

Key to figure ^a	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL			Reference
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	once (G)				250 M (LD50)	Andersen and Mehl 1979
2	Rat (Alderley Park)	once (GO)				1,190 F (LD50)	Clark and Litchfield 1969
3	Rat (Long-Evans)	once (G)				860 (LD50)	Jones et al. 1972

^aThe number corresponds to entries in Figure 2-2.

F = female; (G) = gavage; (GO) = gavage in oil; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level

Figure 2-2. Levels of Significant Exposure to Propylene Glycol Dinitrate – Oral

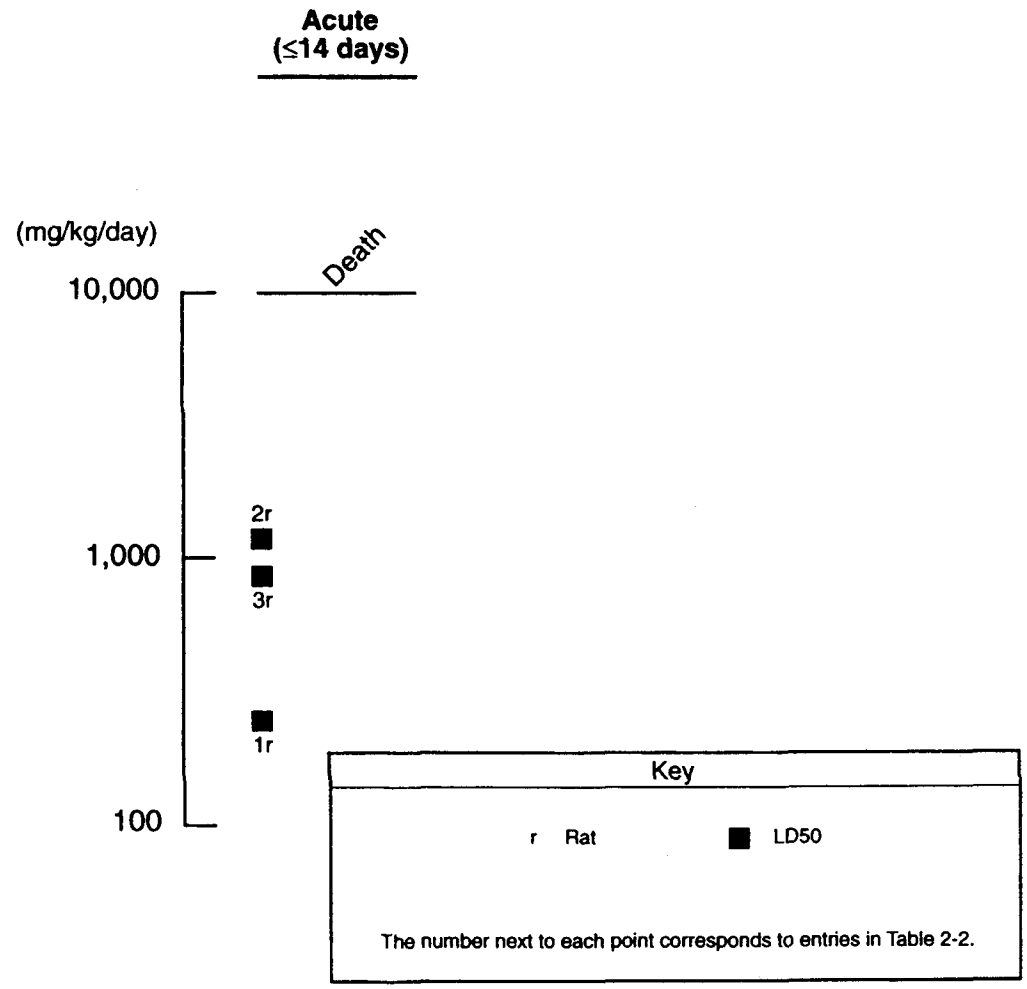


TABLE 2-3. Levels of Significant Exposure to Dibutyl Sebacate - Oral

Key to figure ^a	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (NS)	once (G)				17,200 (LD50)	Komarova 1976, 1979
2	Rat (Sprague-Dawley)	once (NS)				32,000 M (6/6 died)	Smith 1953
3	Mouse (NS)	once (G)				18,100 (LD50)	Komarova 1976, 1979
INTERMEDIATE EXPOSURE							
Reproductive							
4	Rat (Sprague-Dawley)	10 wk ad lib (F)		3,125			Smith 1953
Developmental							
5	Rat (Sprague-Dawley)	10 wk ad lib (F)			3,125	(decreased weight gain of litters)	Smith 1953

TABLE 2-3. Levels of Significant Exposure to Dibutyl Sebacate - Oral (continued)

Key to figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Systemic							
6	Rat (Sprague-Dawley)	2 yr ad lib (F)	Resp	3,125 M			Smith 1953
			Cardio	3,125 M			
			Gastro	3,125 M			
			Hemato	3,125 M			
			Hepatic	3,125 M			
			Renal	3,125 M			
			Endocr	3,125 M			
Immuno/Lymphor							
7	Rat (Sprague-Dawley)	2 yr ad lib (F)		3,125 M			Smith 1953
8	Rat (Sprague-Dawley)	1 yr ad lib (F)		625 M			Smith 1953
Neurological							
9	Rat (Sprague-Dawley)	1 yr ad lib (F)		625 M			Smith 1953

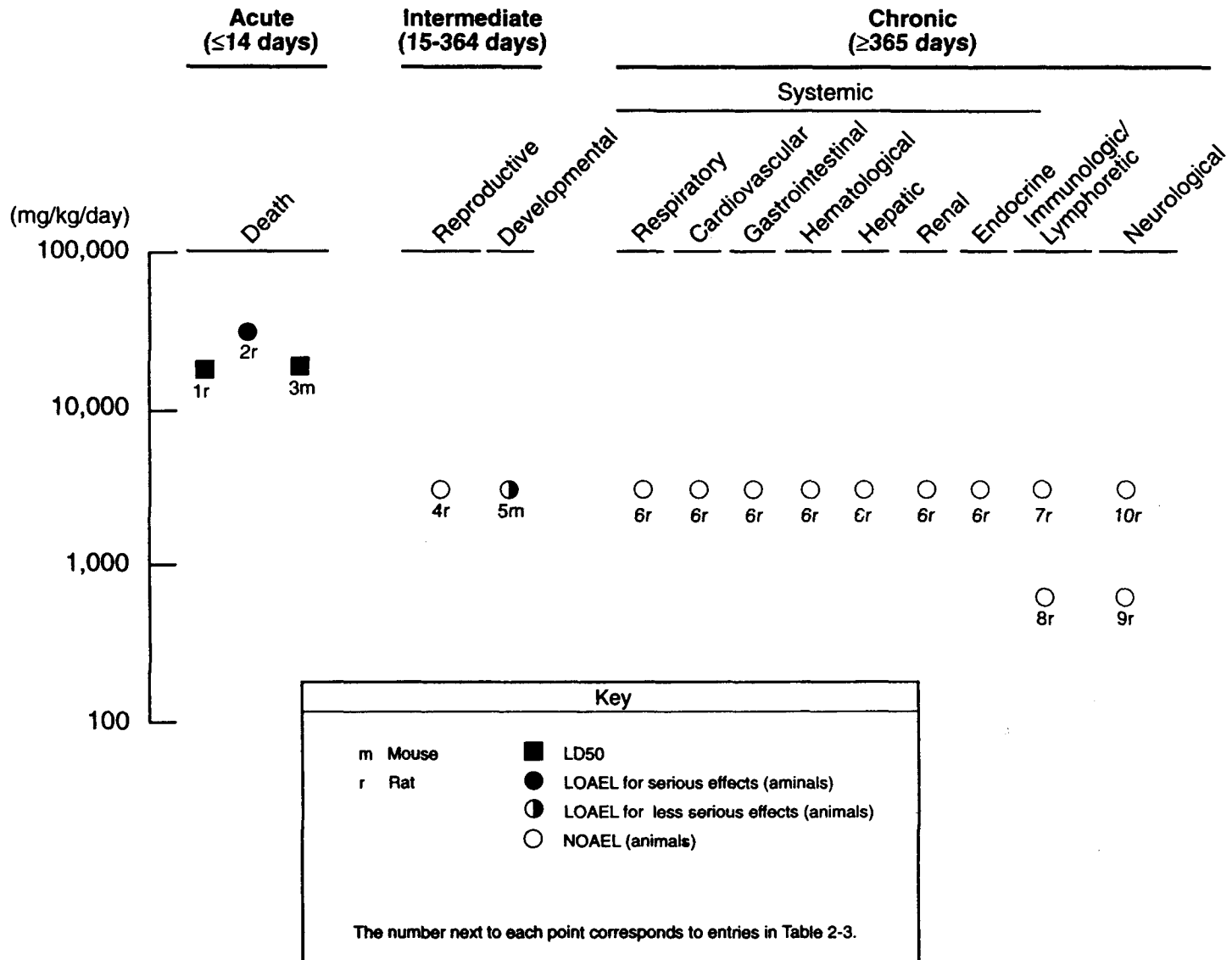
TABLE 2-3. Levels of Significant Exposure to Dibutyl Sebacate - Oral (continued)

Key to figure ^a	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	LOAEL		Reference
				NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	
10	Rat (Sprague- Dawley)	2 yr ad lib (F)		3,125 M		Smith 1953

^aThe number corresponds to entries in Figure 2-3.

ad lib = ad libitum; Cardio = cardiovascular; Endocr = endocrine; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); yr = year(s)

Figure 2-3. Levels of Significant Exposure to Dibutyl Sebacate – Oral



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2.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to Otto Fuel II or its components.

The highest NOAEL values and all reliable LOAEL values for each study for each end point for dibutyl sebacate in rats are recorded in Table 2-3 and plotted in Figure 2-3.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding respiratory effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine. However, necropsy of rats and mice that died as a result of receiving a single oral dose of dibutyl sebacate greater than or equal to 10,000 and 15,000 mg/kg, respectively, revealed hemorrhaging and edema of the lungs (Komarova 1976, 1979). No information regarding the number of animals per dose with lung pathology or the dose relationship of this response was presented. It is possible that the hemorrhaging and edema in the lungs was the result of aspiration of the gastric contents, considering the relatively large gavage volumes used. In contrast, routine gross and histopathological examination of rats consuming 3,125 mg/kg/day of dibutyl sebacate for 2 years demonstrated no treatment-related gross or histopathological alterations in the lungs (Smith 1953).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding cardiovascular effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine. However, cardiovascular end points were reported in animals following oral exposure to dibutyl sebacate in two studies.

Hypervolemia of the heart muscle was observed at necropsy of rats and mice that died following administration of a single oral dose of dibutyl sebacate greater than or equal to 10,000 and 15,000 mg/kg, respectively (Komarova 1976, 1979). Insufficient information regarding the incidence

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of this effect or the number of animals exposed limits this study. Also, it is unclear whether this represents a direct toxic effect of the dibutyl sebacate or is a result of the administration of a large volume of test material. Routine gross and histopathological examination of rats consuming 3,125 mg/kg/day of dibutyl sebacate for 2 years showed no treatment-related gross or histopathological changes in the heart (Smith 1953).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding gastrointestinal effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine. However, gastrointestinal end points were reported in animals following oral exposure to dibutyl sebacate in two studies.

Hypervolemia of the walls of the small intestines and paresis of the stomach were observed in rats and mice that died following oral administration of single doses of dibutyl sebacate greater than or equal to 10,000 and 15,000 mg/kg, respectively (Komarova 1976, 1979). It is unclear whether these effects represented direct toxic effects of the dibutyl sebacate or were the result of administration of a large oral volume of test material. Routine gross and histopathological examination of rats consuming 3,125 mg/kg/day of dibutyl sebacate for 2 years revealed no treatment-related gross or histopathological changes in the stomach or small intestine (Smith 1953).

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding hematological effects in animals after oral exposure to Otto Fuel II or its component, propylene glycol dinitrate. However, limited information was located regarding the hematological toxicity of 2-nitrodiphenylamine and dibutyl sebacate.

An Army review indicated that oral administration of 3,070 mg/kg of 2-nitrodiphenylamine to rats resulted in elevation of methemoglobin levels to 9.45% (Army 1979). (This information was obtained by the U.S. Army through personal communication with American Cyanamid. Because the actual study was unavailable for review, this information could not be verified.)

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Routine hematology performed at 6, 12, 18, and 24 months revealed no effects of consumption of up to 3,125 mg/kg/day of dibutyl sebacate on hemoglobin, total erythrocytes, or total or differential leukocyte counts in rats (Smith 1953). Differential counts taken on bone marrow smears at the time of final sacrifice also revealed no effect on cellular distribution or the myeloid-erythroid ratio.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding hepatic effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine. However, routine gross and histopathological examination of rats consuming 3,125 mg/kg/day of dibutyl sebacate for 2 years revealed no treatment-related gross or histopathological changes in the liver (Smith 1953).

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding renal effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine. However, in two studies, renal end points were evaluated in animals following oral exposure to dibutyl sebacate.

Hypervolemia of the kidneys was observed at necropsy of rats and mice that died as a result of oral administration of a single dose of dibutyl sebacate greater than or equal to 10,000 and 15,000 mg/kg, respectively (Komarova 1976, 1979). However, insufficient information regarding the number of animals tested and the incidence of this observation limits the interpretation of this report. In addition, it is unclear whether this effect is a direct toxic effect of the dibutyl sebacate or the result of the administration of a large volume of test material. Routine gross and histopathological examination of rats consuming 3,125 mg/kg/day of dibutyl sebacate for 2 years revealed no treatment-related gross or histopathological changes in the kidneys (Smith 1953).

Endocrine Effects. Information regarding endocrine effects after oral exposure to Otto Fuel II or its components was limited to an early study with dibutyl sebacate in rats. In that study (Smith 1953), rats fed a diet that provided up to 3,125 mg dibutyl sebacate/kg/day for up to 2 years showed no gross

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or histopathological changes in the thyroid and adrenal glands. No further endocrinological end points were assessed.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after oral exposure to Otto Fuel II or its components propylene glycol dinitrate and 2-nitrophenylamine. However, no gross alterations were observed in the spleens of rats fed a diet that provided 625 mg dibutyl sebacate/kg/day for 1 year or 3,125 mg/kg/day for 2 years (Smith 1953). This information is recorded in Table 2-3 and plotted in Figure 2-3.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding neurological effects in animals after oral exposure to Otto Fuel II or its component, 2-nitrodiphenylamine. However, information regarding the effects of propylene glycol dinitrate and dibutyl sebacate on neurological parameters was available.

A reduced response to external stimuli, prostration, and mild convulsions were observed in rats given single oral doses of propylene glycol dinitrate (Jones et al. 1972). At concentrations ranging from 1,100 to 3,320 mg/kg/day, the effects were observed within 15 minutes, and at lower doses (76-760 mg/kg/day), the symptoms were not as severe and were delayed in onset. The lowest doses at which specific effects were observed in this study were not specified.

Hypervolemia of the "brain substance" was observed in rats and mice that died following administration of a single oral dose of dibutyl sebacate greater than or equal to 10,000 and 15,000 mg/kg, respectively (Komarova 1976, 1979). However, evaluation of this finding is limited because insufficient information regarding the number of animals tested or the dose-related incidence was provided. In addition, it is unclear whether this effect is a direct toxic effect of the dibutyl sebacate or is related to the volume of the test material that was administered. No gross pathological

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changes were observed in the brains of rats consuming 625 mg dibutyl sebacate/kg/day for 1 year or 3,125 mg/kg/day for 2 years (Smith 1953).

The highest NOAEL values for neurological effects in rats following chronic-duration exposure to dibutyl sebacate are recorded in Table 2-3 and plotted in Figure 2-3.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding reproductive effects in animals after oral exposure to propylene glycol dinitrate or 2-nitrodiphenylamine.

The only study located regarding possible reproductive effects of Otto Fuel II was a dominant lethal study in mice which demonstrated that exposure of males to 200 mg/kg/day of Otto Fuel II for 5 days prior to mating had no effect on fertility, the total number of implants per pregnant female, or the number of dead implants per pregnant female (Navy 1982b). However, this study was limited in that the fertility was low overall in both control and treated animals.

Consumption of 3,125 mg/kg/day of dibutyl sebacate in the diet by parental animals for 10 weeks prior to mating caused no adverse effects on fertility, litter size, or litter survival (Smith 1953).

The highest NOAEL for reproductive effects in rats after intermediate-duration oral exposure to dibutyl sebacate is recorded in Table 2-3 and plotted in Figure 2-3.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding developmental effects in animals after oral exposure to Otto Fuel II or two of its components, propylene glycol dinitrate and 2-nitrodiphenylamine.

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Consumption of 3,125 mg/kg/day of dibutyl sebacate in the diet by male and female rats for 10 weeks prior to mating resulted in a decreased weight gain in litters during the preweaning period (Smith 1953). It is unclear whether the reduced growth was caused by contact with test material in utero or during lactation. Additional exposure of a subset of the pups to the parental diet for 21 days postweaning resulted in continued retarded growth. No gross pathological changes were observed at necropsy of the litters following sacrifice at the end of a 21-day postweaning period.

The NOAEL value for developmental effects in rats after intermediate-duration exposure to dibutyl sebacate is recorded in Table 2-3 and plotted in Figure 2-3.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding genotoxic effects in animals after oral exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate.

Otto Fuel II was evaluated for the potential to induce chromosome aberrations in mouse bone marrow cells following the acute oral administration of 20, 62, or 200 mg/kg/day (Navy 1982b). Although the results from the acute study suggested a clastogenic response (i.e., slight but dose-related increases in chromosome damage and the presence of rare complex aberrations at two doses), no definite conclusions could be drawn. In that study (Navy 1982b), treatment with Otto Fuel II did not induce any signs of overt toxicity. Negative results were reported for the mouse dominant lethal assay conducted with comparable acute oral doses of Otto Fuel II (Navy 1982b). (The assay detects mainly chromosome aberrations but may also detect ploidy changes and chromosome nondisjunctions.) The relevance, if any, of the reduced fertility indices in the three treatment groups at the majority of mating intervals (weekly matings for the 7-week test period) could not be determined because similar reduced fertility indices were seen in the historic and concurrent negative controls. Also of concern was the low number of pregnant females (<20/treatment group/mating week) evaluated for critical dominant lethal parameters. Although the dominant lethal indices (i.e., dead implants/total implants) for the majority of treatment groups and mating weeks were within the historical spontaneous range of the reporting laboratory and also within published ranges (Green et al. 1985) the small sample size of

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pregnant females compromised the overall results. It was, nonetheless, concluded that there was no compelling evidence from this study that Otto Fuel II was clastogenic in male mouse germinal cells.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to Otto Fuel II or its components.

No studies were located regarding cancer in animals after oral exposure to Otto Fuel II or its components, propylene glycol dinitrate and 2-nitrodiphenylamine. Limited information was, however, available regarding the tumorigenic potential of dibutyl sebacate in animals after oral exposure. Consumption of up to 3,125 mg/kg/day dibutyl sebacate by rats for 2 years had no effect on tumor incidence (Smith 1953). However, interpretation of this study is limited by the relatively small number of rats tested (20 males and 20 females per dose).

2.2.3 Dermal Exposure

As indicated in the section on inhalation exposure, it is often difficult to clearly separate dermal from inhalation exposures in many occupational studies. Thus, many of the findings from occupational studies described in Section 2.2.1 regarding inhalation exposure are repeated here.

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to Otto Fuel II or its components.

Application of 4,000 mg/kg/day of Otto Fuel II to the skin of pregnant rats during days 6-15 of gestation resulted in the deaths of 25 out of 47 rats at this dose (Cooper et al. 1993). No deaths were reported among pregnant rats at doses as great as 2,000 mg/kg/day or in pregnant rabbits at doses as great as 1,000 mg/kg/day (applied during days 6-18 of gestation) (Cooper et al. 1993).

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Application of propylene glycol dinitrate to the skin of rabbits for 20 days for 2 hours/day resulted in death in 1 of 5 rabbits after the sixth application of 2,000 mg/kg and death in 13 of 14 rabbits after the fifth application of 4,000 mg/kg (Jones et al. 1972). Similarly, 6 of 11 rabbits died as the result of daily application of 3,488 mg/kg for 3 weeks (Andersen and Mehl 1979).

No deaths were reported to result from application of an unspecified amount of 100% dibutyl sebacate to the skin of rabbits for 48 hours (Malette and Von Haam 1952b). This study is limited by the absence of details regarding the sex of the rabbits and dosage of dibutyl sebacate used.

Application of 2-nitrodiphenylamine to the skin of rabbits resulted in a 24-hour dermal LD₅₀ value of >10,000 mg/kg (American Cyanamid 1982). (This information was obtained from a Material Safety Data Sheet on 2-nitrodiphenylamine. Because the actual study was unavailable for review, this value could not be verified.)

All reliable LOAEL values for death in rats following acute-duration dermal exposure to Otto Fuel II are recorded in Table 2-4 and all reliable LOAEL values for death in rabbits after intermediate-duration dermal exposure to propylene glycol dinitrate are recorded in Table 2-5:

2.2.3.2 Systemic Effects

No studies were located regarding gastrointestinal effects in humans or animals after dermal exposure to Otto Fuel II or any of its components.

The highest NOAEL value and all reliable LOAEL values for each end point in rabbits after intermediate-duration dermal exposure to propylene glycol dinitrate are recorded in Table 2-4.

Respiratory Effects. No studies were located regarding respiratory effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, respiratory effects associated with Otto Fuel II exposure have been reported.

Approximately 6% of a group of 87 torpedo maintenance workers with 1-132 months (average 47 months) of occupational exposure to Otto Fuel II reported experiencing dyspnea occasionally during past exposures (Horvath et al. 1981). It is possible that these workers may have been exposed

TABLE 2-4. Levels of Significant Exposure to Otto Fuels II and Components - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
				Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE						
Death						
Rat (Fischer 344)	10 d Gd 6-15				4,000 F (25/47 died)	Cooper et al. 1993
Systemic						
Rat (Fischer 344)	10 d Gd 6-15	Hemato			4,000 F (58% methemoglobinemia)	Cooper et al. 1993
		Derm Bd Wt	2,000 F	4,000 F (moderate erythema)	400 F (25% decrease weight gain in dams)	
Rabbit (New Zealand white)	13 d Gd 6-18	Derm	316 F	1,000 F (marked erythema)		Cooper et al. 1993
		Bd Wt			100 F (38% transient body weight gain decrease in dams)	
Developmental						
Rat (Fischer 344)	10 d Gd 6-15		400 F	2,000 F (decreased fetal weight)	4,000 F (increased resorptions; decreased viable fetuses and number of fetuses per dam)	Cooper et al. 1993
Rabbit (New Zealand white)	13 d Gd 6-18		1,000 F			Cooper et al. 1993

d = day(s); Derm = dermal; Gd = gestation day(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = times

TABLE 2-5. Levels of Significant Exposure to Propylene Glycol Dinitrate - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
				Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE						
Death						
Rabbit (New Zealand white)	20 d 2hr/d				2,000 M (1/5 died)	Jones et al. 1972
Systemic						
Rabbit (New Zealand white)	1-20 d 2hr/d	Resp	1,000 M	2,000 M (rapid, shallow breathing)		Jones et al. 1972
		Cardio		4,000 M (myocardial degeneration)		
		Hemato		1,000 M (transient decrease in hemoglobin)	4,000 M (cyanosis; 35.5% methemoglobin)	
		Hepatic		4,000 M (liver degeneration)		
		Renal		4,000 M (bladder distension; vacuolar change in proximal convoluted tubular epithelium)		
		Bd Wt		4,000 M (11% decrease in final body weight)		
Immuno/Lymphor						
Rabbit (New Zealand white)	1-20 d 2hr/d		4,000 M			Jones et al. 1972
Neurological						
Rabbit (New Zealand white)	20 d 2hr/d		1,000 M	2,000 M (weak appearance)		Jones et al. 1972

TABLE 2-5. Levels of Significant Exposure to Propylene Glycol Dinitrate - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
				Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE						
Death						
Rabbit (New Zealand white)	3 wk 1x/d				3,488 M (6/11 died)	Andersen and Mehl 1979
Systemic						
Rabbit (New Zealand white)	20 d 2hr/d	Dermal		1,000 M (minor skin irritation)		Jones et al. 1972
		Bd Wt	2,000 M			

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s)

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dermally as well as by inhalation (see Section 2.2. I .2) to Otto Fuel II during the maintenance procedures. It is unknown whether the incidence of the dyspnea was increased relative to controls or whether other chemical exposures may have contributed to the dyspnea.

Nasal congestion was reported by approximately 31% of the workers in the study by Horvath et al. (1981). However, this symptom is most likely due to a direct vasodilatory effect of the propylene glycol dinitrate found in Otto Fuel II on the blood vessels of the nasal mucosa and not an effect on respiratory function as such. Therefore, nasal congestion is discussed below as a symptom of a cardiovascular response.

No studies were located regarding respiratory effects in animals after dermal exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, application of 2,000 mg/kg/day of propylene glycol dinitrate to the skin of rabbits for 2 hours/day for 20 days resulted in rapid and shallow breathing (Jones et al. 1972). This effect was apparently not associated with pulmonary damage because histopathologic examination of the lungs of the animals at the termination of exposure found no pathologic alterations, but was probably related to development of methemoglobinemia.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. However, cardiovascular effects have been associated with exposure to Otto Fuel II.

Symptoms identified with nitrate-induced vasodilation or compensatory vasoconstriction have been observed in studies examining human exposure to Otto Fuel II. For example, symptoms that were experienced either frequently or occasionally by 87 torpedo maintenance workers with 1-132 months (average of 47 months) of exposure to Otto Fuel II included headache (65%) and nasal congestion (31%) (Horvath et al. 1981). However, it was not reported whether the incidence of these symptoms was greater than in the controls. Workers at an incinerator at which Otto Fuel II was burned reported that they were able to identify exposures to Otto Fuel II by its characteristic vasodilatory headache (ATSDR 1990). Labile blood pressures were also recorded in the incinerator workers. However, this effect cannot be directly attributed to Otto Fuel II exposure because only 10% of the waste handled at the facility was Otto Fuel II.

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Ten percent of the torpedo maintenance workers also reported experiencing palpitations and 4% reported experiencing chest pain. Although it was not stated whether the incidence of these symptoms was greater than in controls, a study of 1,352 Naval torpedoman's mates with potential exposure to Otto Fuel II demonstrated a significantly greater incidence of hospitalizations for myocardial infarctions or angina pectoris than 14,336 unexposed torpedoman's mates or 29,129, unexposed fire control technicians (Forman et al. 1987). Chest pain and tightness were also reported by several of the incinerator workers, and one worker was diagnosed with nitrate withdrawal angina (ATSDR 1990). However, because of the large number of wastes handled at the facility, other possible causative agents for these cardiac symptoms cannot be eliminated.

No studies were located regarding cardiovascular effects in animals after dermal exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, a single application of 50 mg/kg of propylene glycol dinitrate in corn oil to the skin of rats resulted in a 7-mm Hg fall in blood pressure (Clark and Litchfield 1969). It is unclear whether the decrease in blood pressure is statistically significant. Similar drops in blood pressure were reported in a recent study in which anesthetized rats were applied a wide range of doses (5-450 mg/kg) of propylene glycol dinitrate (Godin et al. 1993). It must be mentioned, however, that the magnitude of the fall in blood pressure was not dose-related. Exposure of rabbits to propylene glycol dinitrate for 2 hours/day for 20 days resulted in the appearance of myocardial degeneration in some rabbits (Jones et al. 1972). It was not specified at which dose (1,000, 2,000, or 4,000 mg/kg) this effect was observed, but it is assumed that it was present at 4,000 mg/kg/day.

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to Otto Fuel II or its components.

No studies were located regarding hematological effects in animals after dermal exposure to 2-nitrodiphenylamine and dibutyl sebacate. However, hematotoxicity was observed in animals following dermal exposure to Otto Fuel II or propylene glycol dinitrate.

A brownish discoloration of the blood was observed at necropsy in pregnant rats that died following application of 4,000 mg/kg/day of Otto Fuel II to the skin during gestation days 6-15 (Cooper et al. 1993). This discoloration was attributed by the authors to be due to methemoglobinemia because a blood sample taken from one of the moribund animals had a methemoglobin level of 56.7%.

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Application of 1,000 mg/kg/day propylene glycol dinitrate to the skin of rabbits for 2 hours/day, for 20 days, resulted in a transient decrease in hemoglobin (Jones et al. 1972). A transient decrease in hematocrit was observed in rabbits receiving 2,000 mg/kg/day in this study. At 2,000 mg/kg, rabbits appeared cyanotic and internal organs appeared dark blue-gray in color. At the highest concentration tested, 4,000 mg/kg/day, methemoglobin levels were elevated to 34.5%.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate. “Seronegative arthritis” was diagnosed in several workers from an incinerator at which 10% of the material burned was Otto Fuel II (ATSDR 1990). However, because of the large number of other wastes processed at the facility, the arthritis cannot be ascribed to Otto Fuel II exposure with any certainty.

No studies were located regarding musculoskeletal effects in animals after dermal exposure to Otto Fuel II or its components.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate. However, transient elevations in unspecified parameters assessing liver function were noted in several incinerator workers at a facility at which approximately 10% of the waste handled was Otto Fuel II (ATSDR 1990). However, because the other wastes incinerated included a number of solvents, this effect cannot be definitively attributed to Otto Fuel II.

No studies were located regarding hepatic effects in animals after dermal exposure to Otto Fuel II and its components, 2-nitrodiphenylamine and dibutyl sebacate. However, hepatic effects were noted in studies in which animals received dermal applications of propylene glycol dinitrate.

Degenerative changes were observed in the livers of rabbits that died after six applications of 4,000 mg propylene glycol dinitrate/kg (Jones et al. 1972). The investigators also indicated that liver oxygen uptake rates and serum proteins, alkaline phosphatase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, isocitrate dehydrogenase, and lactate dehydrogenase were not affected by propylene glycol dinitrate application, but the dose levels referred to (1,000, 2,000, or 4,000 mg propylene glycol dinitrate/kg) were unclear.

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Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to Otto Fuel II or its components.

No studies were located regarding renal effects in animals after dermal exposure to Otto Fuel II or two of its components, 2-nitrodiphenylamine and dibutyl sebacate. However, vacuolar changes were reported in the epithelial cells of the proximal convoluted tubules of the kidneys in a study in which 1,000, 2,000, or 4,000 mg/kg propylene glycol dinitrate was applied to the skin of rabbits for 2 hours/day for 20 days (Jones et al. 1972). However, the doses at which this effect was observed were not reported.

Dermal Effects. No studies were located regarding dermal effects in humans after dermal exposure to propylene glycol dinitrate or 2-nitrodiphenylamine.

Eczema, dermatitis with macropapular rashes, and sweating with no apparent cause were also reported among incinerator workers at a facility at which Otto Fuel II made up approximately 10% of the waste material processed (ATSDR 1990). However, other materials processed at the incinerator could have been responsible for these effects.

Application of undiluted dibutyl sebacate (dosage not reported) to abraded skin of volunteers for an unspecified period of time was not irritating (Askarova and Muryseva 1975). Application of dibutyl sebacate (dosage not reported) to the skin of volunteers for 48 hours also resulted in no irritation (Malette and Von Haam 1952b). No sensitization was observed when the subjects in the latter study were reexposed 2 weeks later.

Animal studies were located regarding dermal effects in animals after dermal exposure to Otto Fuel II and each of its individual components.

Moderate erythema was observed at the application site of pregnant female rats that received a daily dose of 4,000 mg/kg/day of Otto Fuel II for 10 days (Cooper et al. 1993). In addition, marked erythema was observed at the application site of pregnant female rabbits that received a daily dose of 1,000 mg/kg/day of Otto Fuel II for 13 days (Cooper et al. 1993).

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No skin irritation was observed 24 or 72 hours after a single application of an unspecified amount of propylene glycol dinitrate to the skin of rabbits (Jones et al. 1972). Repeated application of 1,000 mg/kg/day to the skin of rabbits for 2 hours/day for 20 days resulted in slight erythema and roughening of the skin that cleared up 5 days after the final application (Jones et al. 1972). At 2,000 mg/kg/day, the skin appeared slightly wrinkled and scaly at the end of the exposure period.

Slight irritation was observed following application of an unspecified dose of dibutyl sebacate to the skin of rabbits and guinea pigs (Komarova 1976, 1979). However, no experimental details were provided in this report. No skin irritation was observed in rabbits following application of an unspecified concentration of dibutyl sebacate to the skin for 48 hours (Malette and Von Haam 1952b). Reexposure 2 weeks later resulted in no sensitization.

Also, no irritation was reported following application of an unspecified amount of 2-nitrodiphenylamine to the skin of rabbits (American Cyanamid 1982). (This information was obtained from a Material Safety Data Sheet on 2-nitrodiphenylamine. Because the actual study was unavailable for review, this lack of irritation could not be verified.)

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to 2-nitrodiphenylamine or dibutyl sebacate.

Occupational exposure to Otto Fuel II resulted in approximately 26% of the 87 workers interviewed complaining of eye irritation as an occasional or frequent exposure-related effect (Horvath et al. 1981). However, the incidence of this effect relative to the control population was not reported. Eye irritation was also a common complaint among incinerator workers at a facility at which Otto Fuel II made up approximately 10% of the waste material processed (ATSDR 1990). However, other materials processed at the incinerator could have been responsible for these effects.

One out of 12 volunteers exposed to 0.2 ppm propylene glycol dinitrate for 8 hours experienced eye irritation, and 8 out of 8 had frank eye irritation after 40 minutes of exposure to 1.5 ppm propylene glycol dinitrate (Stewart et al. 1974). In the same study, no eye irritation was reported in nine subjects exposed to 0.2 ppm propylene glycol dinitrate 8 hours/day for 5 days (Stewart et al. 1974).

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Data in animals are limited to propylene glycol dinitrate and 2-nitrodiphenylamine. Conjunctival redness was observed 5 minutes after an unspecified dose of propylene glycol dinitrate was instilled into the eyes of rabbits (Jones et al. 1972). The irritation disappeared within 24 hours. No effects on the cornea or iris were observed.

Also, no irritation was reported following application of an unspecified amount of 2-nitrodiphenylamine to the eyes of rabbits (American Cyanamid 1982). (This information was obtained from a Material Safety Data Sheet on 2-nitrodiphenylamine. Because the actual study as unavailable for review, this value could not be verified.)

Body Weight Effects. The only information regarding body weight effects after dermal exposure to Otto Fuel II or its components is that provided by Cooper et al. (1993) who reported significant decreases in body weight gain in both rats and rabbits after repeated applications of Otto Fuel II to the skin during pregnancy (400 mg/kg/day to rats, 100 mg/kg/day to rabbits). However, food consumption data were not provided.)

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after dermal exposure to propylene glycol dinitrate and 2-nitrodiphenylamine. However, sensitization was tested following dermal exposure to dibutyl sebacate and immunological effects (possibly related to Otto Fuel II exposure) were reported.

No skin sensitization was observed in volunteers exposed to an unspecified amount of 100% dibutyl sebacate 2 weeks following an initial 2-day dermal exposure (Malette and Von Haam 1952b). However, in workers exposed to lubricants containing dibutyl sebacate, a 3.3% rate of sensitization was observed when nonirritating concentrations of dibutyl sebacate were applied to their skin (Askarova and Muryseva 1975). Nonirritating concentrations were determined in volunteers with no known exposure to the lubricants or their ingredients. This study is limited, however, in that a control group was not used in the sensitization portion of the study.

A child of a worker at an incineration facility at which Otto Fuel II was processed developed asthma (ATSDR 1990). The asthma was linked to materials handled at the incinerator because when the

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father of the child stopped bringing his work clothing home, the child's condition improved. However, Otto Fuel II comprised only approximately 10% of the wastes handled at the facility; thus, other materials may have triggered the reaction in the child. The possibility also exists that the asthma-like response may have been caused by tetryl particles activating nerve receptors which triggered vagal reflexes inducing bronchoconstriction.

No studies were located regarding immunological effects in animals after dermal exposure to Otto Fuel II or its component, 2-nitrodiphenylamine. However, extremely limited information was located regarding immunological effects of propylene glycol dinitrate and dibutyl sebacate in animals after dermal exposure. No studies that directly examined immunological function were located. However, total and differential leukocyte counts and routine gross and histopathological examination of the spleen were normal in rabbits after application of doses as high as 4,000 mg/kg/day of propylene glycol dinitrate to the skin for 2 hours/day for 20 days (Jones et al. 1972).

No skin sensitization was observed in rabbits reexposed to an unspecified dose of 100% dibutyl sebacate 2 weeks after a 2-day dermal application (Malette and Von Haam 1952b).

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate.

The only reliable information regarding neurological effects in humans after dermal exposure to Otto Fuel II was found in a study examining the effects of exposure to Otto Fuel II during torpedo maintenance procedures on tests of balance and oculomotor performance (Horvath et al. 1981). Twenty-nine workers with an unspecified number of months of previous occupational exposure to Otto Fuel II were tested both before and after a typical torpedo maintenance procedure. The duration of the procedure was between 30 and 45 minutes, and both inhalation and dermal exposures were likely. The inhalation exposures ranged from 0 to 0.22 ppm, but the amount of dermal exposure was not monitored. No significant difference in the tests evaluating balance and coordination were observed when comparing pre- and postexposure results. However, a significant decrease in saccade velocity and increase in saccade delay were observed. Although these effects were reported by the authors to be functionally insignificant, they were proposed as evidence of preclinical neurological effects of Otto

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Fuel II exposure. Eighty-seven torpedo maintenance workers were also questioned regarding effects experienced during past exposures. Dizziness was reported by 13% of the workers and loss of balance was reported by 1% of the workers; however, the incidence of these effects was not compared to that in an unexposed control group.

Workers at an incinerator where Otto Fuel II comprised approximately 10% of the waste materials processed, were diagnosed with a number of neurological disorders (ATSDR 1990). These included mood disorders, cerebellar dysfunction (ataxia, tremor, nystagmus), cognitive disorders, memory loss, and possible vestibular dysfunction. Magnetic resonance imaging showed that two of the four workers, interviewed in the report had cerebellar and cortical atrophy. However, the effects observed cannot be attributed to Otto Fuel II with certainty because a large number of solvents and other wastes were also handled at the incinerator. It is also unclear from the report whether the individuals that were examined represented a substantial percentage of the workers that were exposed to Otto Fuel II.

Headaches have frequently been reported by workers occupationally exposed to Otto Fuel II (Horvath et al. 1981). However, the headaches are most likely associated with meningeal blood vessel dilation (Nickerson 1975) (see also Cardiovascular Effects in Section 2.2.3.2).

No studies were located regarding neurological effects in animals after dermal exposure to Otto Fuel II or its components, 2-nitrodiphenylamine or dibutyl sebacate. Extremely limited information was obtained regarding possible neurological effects resulting from dermal application of propylene glycol dinitrate. Weakness was observed in rabbits after the third 2-hour/day application of 2,000 mg/kg/day of propylene glycol dinitrate to the skin (Jones et al. 1972). The animals had recovered by the 20th daily application. However, it is not known whether the weakness that was observed was neurological in origin. The gross and histopathological examination of sections of brain and spinal cord showed no adverse structural effects of propylene glycol dinitrate on these tissues.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to Otto Fuel II or any of its individual components.

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2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate. However, a child with multiple birth defects was born to the wife of a worker at an incinerator where Otto Fuel II comprised approximately 10% of the waste processed (ATSDR 1990). The defects included club foot, imperforate anus, renal anomalies, and persistence of the cloaca. Chromosomal studies on the child were normal. The birth defects cannot be definitively ascribed to exposure to Otto Fuel II because other possible causes for the defects exist (i.e., exposure to other developmental toxicants) and the incidence of similar defects in a control population were not adequately characterized.

No studies were located regarding developmental effects in animals after dermal exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate.

Rat pups from dams receiving daily dermal applications of 2,000 mg/kg/day of Otto Fuel II during gestation days 6-15 had decreased body weight when compared to pups from control rats (Cooper et al. 1993). At 4,000 mg/kg/day, an increase in the number of resorptions, decrease in the number of viable pups, and decreases in pup weight were observed. Decreased maternal body weights were observed among dams at 2,000 and 4,000 mg/kg/day indicating maternal toxicity at these doses. Discoloration of the blood, likely due to methemoglobinemia, was observed in dams at the 4,000 mg/kg/day dose. No developmental toxicity was observed in pups from dams at doses where no maternal toxicity was evident. No increase in visceral or skeletal abnormalities was observed at any dose. No developmental toxicity was observed at doses as high as 1,000 mg/kg/day in rabbits that received daily dermal applications of Otto Fuel II during gestation days 6-18 (Cooper et al. 1993).

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to Otto Fuel II or its components.

Genotoxicity studies are discussed in Section 2.4.

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2.2.3.8 Cancer

No studies were Located regarding cancer in humans or animals after dermal exposure to Otto Fuels XI or its components.

2 . 3 TOXICOKINETICS

Otto Fuel II is a mixture of three components. Discussion of the toxicokinetics of Otto Fuel II is directed specifically at the toxicokinetics of the individual components of Otto Fuel II. This has been done because the individual components of Otto Fuel II, and not the mixture itself, are subject to absorption, distribution, metabolism, and excretion by the body based upon their individual physical and chemical characteristics.

Data regarding the toxicokinetics of the components of Otto Fuel II in humans are limited to a single study in volunteers exposed to propylene glycol dinitrate by the inhalation route. These data provide qualitative evidence that propylene glycol dinitrate may be absorbed in humans by this route. There are no data regarding oral or dermal absorption of 2-nitrodiphenylamine or dibutyl sebacate in humans. There is qualitative evidence that animals can absorb propylene glycol dinitrate after inhalation, oral, and dermal exposure, and also, that animals can absorb 2-nitrodiphenylamine and dibutyl sebacate after oral exposure. The mechanisms by which the components of Otto Fuel II are transported to the tissues are unknown. There are no data regarding distribution patterns for the components of Otto Fuel II or their metabolites in humans or animals. No information is available regarding the metabolism of the components of Otto Fuel II in humans. In animal studies, limited data suggest that propylene glycol dinitrate undergoes enzymatic reduction to the mononitrates and nitrite, with the subsequent formation of inorganic nitrate. No data were available for 2-nitrodiphenylamine or dibutyl sebacate. There is no information on how 2-nitrodiphenylamine or dibutyl sebacate or their potential metabolites are excreted in humans or animals. Qualitative information indicates that humans may excrete unchanged propylene glycol dinitrate in expired air after inhalation exposure and that urinary nitrate may represent a major excretion for propylene glycol dinitrate in animals after dermal exposure. The mechanism of 2-nitrodiphenylamine or dibutyl sebacate toxicity is unknown. The toxicity of propylene glycol dinitrate is related to its vasodilating properties and its methemoglobin forming capacity in the red blood cells.

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2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located that examined the absorption of the nonvolatile components of Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate, in humans after inhalation exposure.

Limited information exists regarding absorption of the Otto Fuel II component, propylene glycol dinitrate, in humans as a result of inhalation exposures. Less than 5 ppb (limit of detection of the method) of propylene glycol dinitrate were measured in the blood of volunteers during a 3.2-hour exposure to test atmospheres containing 1.5 ppm propylene glycol dinitrate (Stewart et al. 1974), indicating that absorption occurs during inhalation exposures.

No studies were located that examined the absorption of two of the components of Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate, in animals after inhalation exposure.

Data are also extremely limited regarding absorption of the Otto Fuel II component, propylene glycol dinitrate, in animals as a result of inhalation exposures. Exposure of monkeys to concentrations of 1.6 ppm and 4.2 ppm of propylene glycol dinitrate for 20 and 14 days, respectively, resulted in the detection of small amounts of propylene glycol dinitrate in plasma (Mattsson et al. 1981). During exposures to 1.6 ppm, 35 ug/mL propylene glycol dinitrate was detected in plasma, and during exposures at 4.2 ppm, 170 ug/mL was detected in plasma.

2.3.1.2 Oral Exposure

No studies were located that examined the absorption of the components of Otto Fuel II, propylene glycol dinitrate, 2-nitrodiphenylamine, dibutyl sebacate, in humans after oral exposure.

No quantitative data regarding oral absorption of the components of Otto Fuel II, propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate, were located. However, all three components have been demonstrated to cause some measure of toxicity after ingestion (American Cyanamid 1982; Andersen and Mehl 1979; Clark and Litchfield 1969; Jones et al. 1972; Smith 1953), indicating some gastrointestinal absorption.

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2.3.1.3 Dermal Exposure

No studies were located that examined the absorption of the components of Otto Fuel II, propylene glycol dinitrate, 2-nitrodiphenylamine, dibutyl sebacate, in humans after dermal exposure.

Also, no studies were located that either directly or indirectly examined the absorption of two of the components of Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate, in animals after dermal exposure. Information supporting the dermal absorption of propylene glycol dinitrate was obtained from studies demonstrating systemic toxicity after dermal exposure in rats (Clark and Litchfield 1969) and rabbits (Jones et al. 1972) and from a recent study which attempted to quantify absorption in rats (Godin et al. 1993). For example, a transient decrease in hemoglobin was observed following dermal application of doses of propylene glycol dinitrate as low as 1,000 mg/kg/day, 2 hours/day, for 20 days to rabbits (Jones et al. 1972). Also, observation of elevated levels of the metabolite nitrate in the urine of rabbits receiving a dermal dose of 4,000 mg/kg/day supported the absorption of propylene glycol dinitrate after dermal exposure (Jones et al. 1972). In a recent study in rats (Godin et al. 1993), dermal absorption of propylene glycol dinitrate was estimated as the difference between the amount of compound applied and the amount that could be later extracted from the skin. It should be noted, however, that this difference may not necessarily indicate absorption since local metabolism may occur. The authors found that, in general, absorption was inversely related to the amount applied, such that 75% of a 5 mg/kg dose was presumably absorbed, whereas only 20% of a 450 mg/kg dose was absorbed.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Extremely little information is available regarding the distribution of the components of Otto Fuel II following inhalation exposure in humans. No information was located regarding the distribution of 2-nitrodiphenylamine or dibutyl sebacate. The only information located regarding the distribution of propylene glycol dinitrate in humans was the observation that propylene glycol dinitrate could be measured in the blood of volunteers during inhalation exposures to propylene glycol dinitrate volatilized from Otto Fuel II (Stewart et al. 1974).

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Similarly, no information was located regarding the distribution of 2-nitrodiphenylamine or dibutyl sebacate in animals after inhalation exposure. Also, no information was located regarding a differential distribution of propylene glycol dinitrate in animals after inhalation exposure. The only information located regarding the distribution of propylene glycol dinitrate in animals was the observation that propylene glycol dinitrate could be measured in plasma of monkeys during inhalation exposures to concentrations of 1.6 and 4.2 ppm (Mattsson et al. 1981). In vitro studies using dog blood have shown that propylene glycol dinitrate preferentially associates with red blood cells in the blood (Air Force 1982b). The ratio of propylene glycol dinitrate in red blood cells versus plasma was 2.12.

2.3.2.2 Oral Exposure

No information was located regarding the distribution of the components of Otto Fuel II in humans or animals after oral exposure.

2.3.2.3 Dermal Exposure

No information was located regarding the distribution of the components of Otto Fuel II in humans or animals after dermal exposure.

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

No information was located regarding the metabolism of the components of Otto Fuel II in humans after inhalation exposure.

No studies were located that examined the metabolism of either 2-nitrodiphenylamine or dibutyl sebacate in animals after inhalation exposure. Also, no studies were located that followed the metabolism of propylene glycol dinitrate in animals after inhalation exposure. The only information relating to the metabolism of propylene glycol dinitrate after inhalation exposure was the observation of elevated blood levels of inorganic nitrate, a metabolic product of propylene glycol dinitrate, in dogs and monkeys exposed to 35 ppm for 24 hours/day for 90 days (Jones et al. 1972). Nitrate is not

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specific to propylene glycol dinitrate, however; thus, an increase in urinary nitrates is not specific for propylene glycol dinitrate exposure.

2.3.3.2 Oral Exposure

No information was located regarding the metabolism of the components of Otto Fuel II in humans or animals after oral exposure.

2.3.3.3 Dermal Exposure

No information was located regarding the metabolism of the components of Otto Fuel II in humans or animals after dermal exposure.

2.3.3.4 Other Routes of Exposure

No information was located on the metabolism of the components of Otto Fuel II in humans exposed by other routes.

Also, no information was located on the metabolism of 2-nitrodiphenylamine in animals exposed by other routes. The metabolism of propylene glycol dinitrate has been studied in animals after parenteral administration (Clark and Litchfield 1969). Subcutaneous injection of 65 mg/kg propylene glycol dinitrate in rats caused a rapid increase in blood levels of propylene glycol dinitrate. Peak blood levels of propylene glycol dinitrate were observed within 1 hour and then were observed to decline to zero by 8-12 hours postinjection. Appearance of metabolites of propylene glycol dinitrate in the blood was maximal 2-4 hours postinjection and had declined to essentially zero by 8-12 hours postinjection. The predominant metabolite observed in the blood was nitrate, with propylene glycol 2-mononitrate observed at approximately one-half the concentration of inorganic nitrate. The levels of propylene glycol 1-mononitrate and inorganic nitrite were less than half that of propylene glycol 2-mononitrate. Similar results have been reported in rats after intravenous injection of 0.1-30 mg propylene glycol dinitrate/kg (Godin et al. 1994). In that study, maximum concentration of metabolites (propylene glycol 2-mononitrate and propylene glycol I-mononitrate) in blood was achieved 10 minutes after the injection. Elimination of the parent compound from blood was monoexponential. For doses of 0.3, 3, and 30 mg/kg, the elimination half-time of propylene glycol dinitrate was 8.8, 13.1, and 17.4 hours,

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respectively. Rapid metabolism of propylene glycol dinitrate *in vitro* has also been observed. Incubation of propylene glycol dinitrate in rat blood resulted in degradation of approximately half of the propylene glycol dinitrate by 60 minutes and degradation of half of the remainder by 120 minutes (Clark and Litchfield 1969). Levels of inorganic nitrate and the mononitrates increased with time, but only small amounts of inorganic nitrite were observed. The breakdown of propylene glycol dinitrate *in vitro* was shown to occur primarily in the erythrocytes, as no degradation in purified plasma was observed (Clark and Litchfield 1969). In contrast with the *in vivo* situation, however, the mononitrates were stable and did not undergo further degradation. Based on these observations and their study of the metabolism of ethylene glycol dinitrate (Clark and Litchfield 1967) the study authors proposed the following metabolic scheme (Figure 2-4): A nitrate group of propylene glycol dinitrate could be reduced to give an organic nitrite-nitrate, followed by hydrolysis to yield the mononitrate and inorganic nitrite. The inorganic nitrite in the blood could then be oxidized to inorganic nitrate. The authors suggested that the predominance of the 2-isomer in the blood may have been due to either its more rapid formation or the more rapid degradation of the 1-isomer.

The only information regarding metabolism of dibutyl sebacate was an *in vitro* analysis in which dibutyl sebacate was added to a preparation of pancreatic lipase (Smith 1953). Hydrolysis of dibutyl sebacate in the lipase preparation was reported to occur more rapidly than the hydrolysis of triolein, causing the author to speculate that dibutyl sebacate is metabolized in the body by the same pathways used in normal fat metabolism. No other information on the metabolism of dibutyl sebacate was located.

2.3.4 Excretion

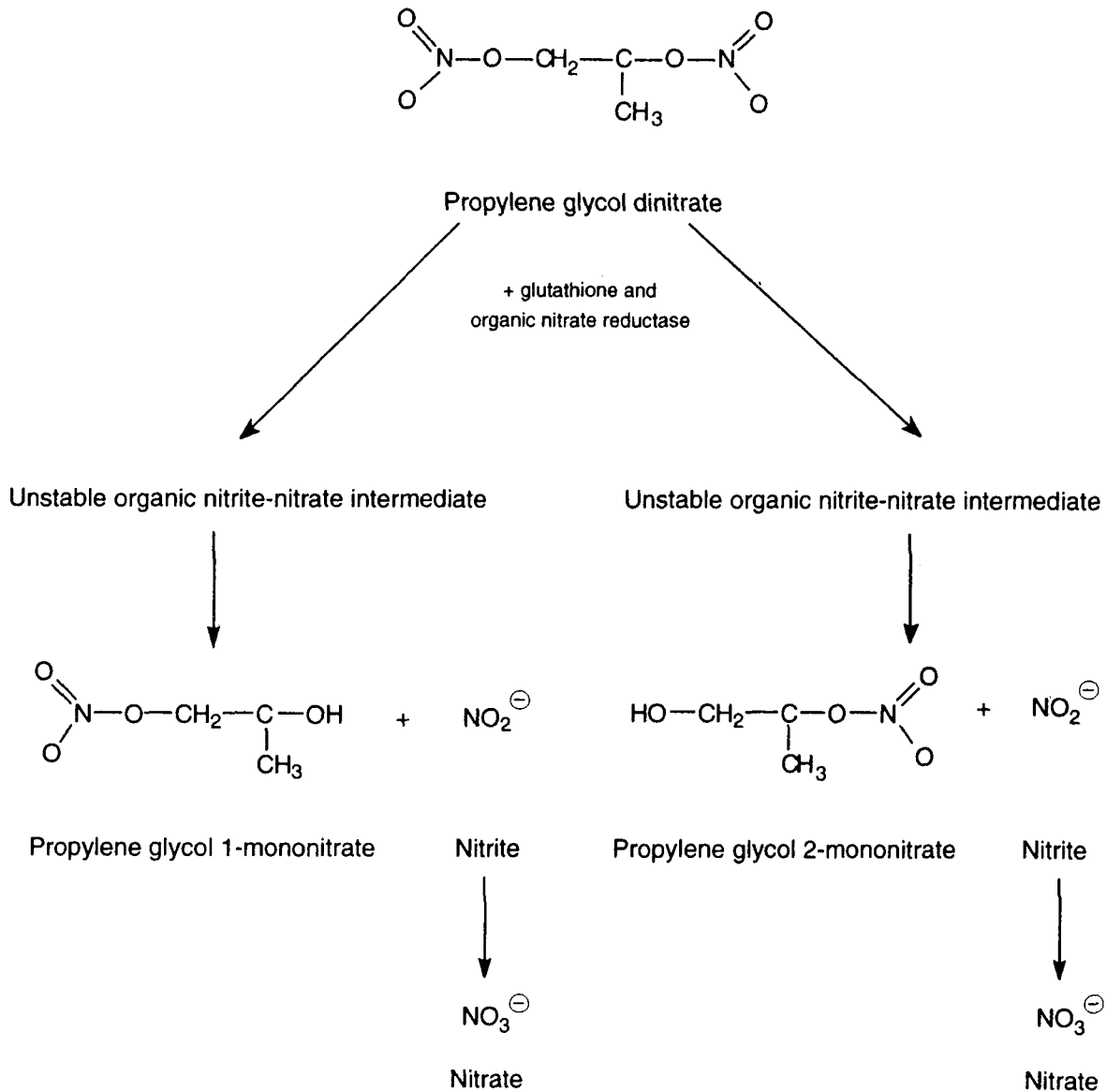
2.3.4.1 Inhalation Exposure

No studies were located that examined the excretion of 2-nitrodiphenylamine or dibutyl sebacate in humans after inhalation exposure.

Alveolar breath samples taken from volunteers during and after exposure to 1.5 ppm propylene glycol dinitrate demonstrated the short duration of excretion of this chemical in expired air (Stewart et al. 1974). During exposure, alveolar breath samples contained between 20 and 35 ppb propylene glycol dinitrate. By 5 minutes after the termination of exposure, only between 1 and 5 ppb of propylene

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FIGURE 2-4. Proposed Metabolic Pathway for Propylene Glycol Dinitrate *



As proposed by Clark and Litchfield 1969

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glycol dinitrate were found in expired air. No measurable propylene glycol dinitrate was found in expired air 15 minutes after the termination of exposure. No information on other routes of excretion of propylene glycol dinitrate in humans was located.

No studies were located that examined the excretion of any of the components of Otto Fuel II in animals after inhalation exposure.

2.3.4.2 Oral Exposure

No studies were located that examined the excretion of any of the components of Otto Fuel II in humans or animals after oral exposure.

2.3.4.3 Dermal Exposure

No studies were located that examined the excretion of any of the components of Otto Fuel II in humans after dermal exposure.

No studies were located regarding the excretion of 2-nitrodiphenylamine or dibutyl sebacate in animals after dermal exposure. Only extremely limited information was found regarding excretion of propylene glycol dinitrate in animals after dermal exposure. One study reported that excretion of nitrate in the urine of rabbits exposed to 4,000 mg/kg/day for 2 hours/day for 20 days accounted for approximately 7% of nitrate in the propylene glycol dinitrate that had been applied (Jones et al. 1972). Because excess propylene glycol dinitrate was wiped off each day at the end of the 2-hour exposure period, the excretion of nitrate relative to the applied dose of propylene glycol dinitrate is unknown.

2.3.4.4 Other Routes of Exposure

No information was located on the excretion of the components of Otto Fuel II in humans exposed by other routes.

No information was located on the excretion of 2-nitrodiphenylamine or dibutyl sebacate in animals exposed by other routes. Subcutaneous injection of a dose of 65 mg propylene glycol dinitrate/kg in the rat resulted in rapid excretion of unmetabolized propylene glycol dinitrate and its metabolites in

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the urine (Clark and Litchfield 1969). Urinary excretion of propylene glycol dinitrate and its metabolites was essentially complete within 24 hours. Inorganic nitrate was the major metabolite excreted in the urine, accounting for approximately 56% of the nitrate in the injected dose. Unmetabolized propylene glycol dinitrate and other metabolites were present in the urine in amounts less than 0.5% of the injected dose. Excretion of unmetabolized propylene glycol dinitrate in the urine accounted for less than 1% of an intravenous dose (Air Force 1982b). In addition, less than 1% of a dose of propylene glycol dinitrate administered intravenously was excreted unchanged in expired air (Air Force 1982b). The major means of elimination of propylene glycol dinitrate from the blood was by metabolism through stepwise removal of nitrate radicals. The rate constant for elimination by metabolism was estimated as 0.895 min⁻¹ (Air Force 1982b).

2.3.5 Mechanisms of Action

No information was located regarding the mechanism by which propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate enter the blood stream from the lungs, skin, or gastrointestinal tract. The mechanism of propylene glycol dinitrate toxicity is related to its vasodilating capacity. Propylene glycol dinitrate is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates such as nitroglycerin, which is widely used for the symptomatic treatment of angina pectoris (Nickerson 1975). Organic nitrates induce peripheral vasodilation, decreased ventricular ejection time, relaxation, and a longer period of coronary blood flow. One of the earliest consequences of overexposure to propylene glycol dinitrate (or to Otto Fuel II) is a vasodilation of the cerebral vessels, which is believed to be the major factor in the development of the typical "trinitrotoluene" headache. Should the overexposure be more severe, the relaxation of the vascular smooth muscle can result in a fall in blood pressure followed by a compensatory vasoconstriction (Abrams 1980). However, a decrease in the magnitude of the vasodilating effect has been observed after repeated exposure to organic nitrates. Although the exact mechanism of initiation and maintenance of tolerance to organic nitrates is not known, several possibilities have been suggested, including depletion of sulfhydryl groups at the receptor sites, reduced availability or activity of the active intermediate S-nitrosothiol, and altered pharmacokinetics leading to decreased nitrate concentration in vascular tissues (Parker 1985). Massive overexposure to propylene glycol dinitrate can produce toxic levels of methemoglobin. This property is shared by many organic and inorganic nitrates and also by aromatic amines, 2-nitrodiphenylamine among them (Beard and Noe 1981; Donovan 1990; Ellenhom and Barceloux 1988). Methemoglobinemia is defined

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as a methemoglobin concentration of greater than 1%, and it results from iron in the normal ferrous state being oxidized to the ferric state at a rate that exceeds the erythrocyte's reducing capacity. 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. The possible mechanism of dibutyl sebacate toxicity is not known.

2.4 RELEVANCE TO PUBLIC HEALTH

Exposure to Otto Fuel II may occur by the inhalation, oral, or dermal routes. The only component of Otto Fuel II with significant volatility is propylene glycol dinitrate. Thus, inhalation exposures to Otto Fuel II would consist primarily of inhalation exposure to propylene glycol dinitrate. Oral exposures to all three components as well as the mixture, Otto Fuel II, are possible through consumption of contaminated water. Information was located that indicated that propylene glycol dinitrate and 2-nitrodiphenylamine are released into waste water effluent streams from plants manufacturing and/or using these substances as a military propellant. It is unknown whether oral exposure to propylene glycol dinitrate and 2-nitrodiphenylamine may occur through consumption of contaminated food products because extremely limited data are available on their presence in food products or their bioaccumulation, biomagnification, or biodegradation. It is likely that significant ingestion of dibutyl sebacate may occur as a result of its civilian use in food packaging materials and as a flavor enhancer in ice cream, candy, baked goods, and non-alcoholic beverages. It is unknown to what extent dermal exposure to Otto Fuel II and its components is likely through contact with contaminated soils because information on the extent of soil contamination, leaching, and/or soil adsorption of these substances was not located. Limited information was also located regarding the degradation of the components in the environment, but the available data indicate that degradation would occur fairly rapidly (i.e., within days), It is likely that dermal contact with dibutyl sebacate may be significant as a result of the civilian use of this substance (as a lubricant in shaving lotions).

The group with the greatest likelihood of exposure to Otto Fuel II consists of persons with occupational exposure to torpedo propellants as this is the predominant use of Otto Fuel II. Information on the effects that occur in humans comes from studies of workers occupationally exposed to Otto Fuel II and from experimental exposures of humans to propylene glycol dinitrate and dibutyl sebacate. These studies indicate that short-term exposure to Otto Fuel II may result in headache, loss

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of balance, poor eye-hand coordination, eye irritation, and dizziness, and that propylene glycol dinitrate is probably the component of Otto Fuel II responsible for these effects. Studies of longer-term exposure suggest that humans may experience an increased incidence of angina and myocardial infarctions.

For the most part, studies in animals support the observation of these toxic effects. In addition, animal studies indicate that methemoglobinemia, degenerative hepatic and renal effects, and developmental toxicity (at maternally toxic doses) may occur.

Minimal Risk Levels for Otto Fuel II and Its Components.

Inhalation MRLs.

- An MRL of 0.003 ppm has been derived for acute inhalation exposure (14 days or less) to propylene glycol dinitrate.

The acute inhalation MRL was based on a NOAEL for altered visual evoked responses in volunteers exposed to concentrations ranging from 0.03 to 1.5 ppm for 1-8 hours (Stewart et al. 1974). At 0.2 ppm 7 of 9 subjects reported headaches. The change in visual evoked response was duration-related indicating a cumulative effect. Exposure to 0.5 ppm for 8 hours resulted in nausea, dizziness, and more markedly altered visual evoked responses. These results are supported by other studies in humans (Horvath et al. 1981) and studies in monkeys (Mattsson et al. 1981).

- An MRL of 0.00004 ppm has been derived for intermediate inhalation exposure (15-364 days) to propylene glycol dinitrate.

The intermediate inhalation MRL was based on a LOAEL for hematological changes in dogs exposed to 0.2 ppm propylene glycol dinitrate intermittently for 14 months (Air Force 1985a). As early as 4 weeks into the study, significantly decreased hematocrit, hemoglobin, red blood cells, and reticulocytes were observed, and by exposure day 44, significantly increased methemoglobin levels were observed. No other exposure level was tested. These results are supported by human data (Donovan 1990), and also by studies in other animal species, such as guinea pigs, rats, and monkeys (Jones et al. 1972).

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- An MRL of 0.00004 ppm has been derived for chronic inhalation (365 days or more) to propylene glycol dinitrate.

The chronic inhalation MRL was based on a LOAEL for hematological changes in dogs exposed to 0.2 ppm propylene glycol dinitrate intermittently for 14 months (Air Force 1985a). Significant hematological changes, such as decreased hematocrit, hemoglobin, red blood cells, and reticulocytes, and increased in methemoglobin concentration were observed before the seventh week of exposure. These effects persisted at the termination of the study at 14 months. Methemoglobinemia was also reported in rats exposed to propylene glycol dinitrate for 1 year (Air Force 1985a).

Inhalation MRLs were not derived for Otto Fuel II due to lack of proper exposure characterization in the human studies and lack of animal data. Inhalation MRLs were not derived for 2-nitrodiphenylamine, or dibutyl sebacate due to both lack of human and animal data.

Oral MRLs.

No oral MRLs were derived for Otto Fuel II or its components due to lack of adequate human and animal data. An intermediate and a chronic duration study were identified for oral exposure to dibutyl sebacate (Smith 1953); however, the fact that this early study represents the only existing information on dibutyl sebacate precludes its use as the basis for MRLs.

Dermal MRLs were not derived because of the lack of an appropriate methodology for doing so.

Death. No studies were located that reported deaths in humans attributable to exposure to Otto Fuel II or any of its components. Studies in animals indicate that there are species differences in lethality of propylene glycol dinitrate by the inhalation route. Rabbits appear most sensitive with deaths reported after continuous exposure to concentrations as low as 35 ppm (Jones et al. 1972). Animal studies also indicate that propylene glycol dinitrate is the most toxic component of Otto Fuel II following exposure by the oral route. Oral LD₅₀ values in rats for propylene glycol dinitrate ranged from 250 to 1,190 mg/kg (Andersen and Mehl 1979; Clark and Litchfield 1969; Jones et al. 1972), whereas the oral LD₅₀ values in rats for 2-nitrodiphenylamine and dibutyl sebacate were 6,150 (Army 1979) and 17,200 mg/kg (Komarova 1976, 1979), respectively. Comparison of the dermal lethality of the individual components of Otto Fuel II in rabbits is not as straightforward because of differing

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durations of exposure and incomplete information on the doses applied; however, the same hierarchy of toxicity appears to hold (Army 1979; Jones et al. 1972; Mallette and Von Haam 1952b). Very limited information is available on the cause of death in these studies, but symptoms observed in propylene glycol dinitrate-treated animals prior to death are consistent with methemoglobin-induced anoxia (Jones et al. 1972).

Although environmental data are limited, based on the potentially rapid degradation of the components of Otto Fuel II and the high doses necessary to cause death, it is unlikely that propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate levels near hazardous waste sites are sufficient to cause death in exposed populations.

Systemic Effects.

Respiratory Effects. A small percentage of workers with occupational exposure to Otto Fuel II have reported experiencing dyspnea during occupational exposure to this substance (Horvath et al. 1981); however, the majority of animal studies have not reported similar effects, and those that have, do not clearly demonstrate the cause for this symptom. Application of a large concentration of propylene glycol dinitrate to the 'skin of rabbits (2,000 mg/kg/day) has been reported to cause rapid and shallow breathing (Jones et al. 1972), but examination of the respiratory system at necropsy did not reveal any abnormalities that might account for the change in breathing pattern. Chronic inhalation exposure of rats to 35 ppm propylene glycol dinitrate resulted in microscopic evidence of slight respiratory irritation (mild degeneration of the nasal epithelium and very slight pulmonary inflammation) (Air Force 1985a); however, it is unlikely that these findings are associated with the dyspnea reported by exposed workers. Dyspnea has been reported to be a common finding in patients with clinically significant methemoglobinemia (see Hematological Effects below) (Bronstein and Currance 1988); thus, the dyspnea described in the above reports may have been associated with methemoglobinemia. The combined results of these studies indicate that sensitive members of exposed populations near hazardous waste sites may experience difficulty breathing when exposed to sufficiently high concentrations, but the cause of this effect remains unknown.

Cardiovascular Effects. The major component of Otto Fuel II, propylene glycol dinitrate, is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates such as amyl nitrate and nitroglycerin. The primary cardiovascular action of the therapeutic nitrates is to relax

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vascular smooth muscle resulting in a fall in blood pressure. Under normal conditions, this decrease in blood pressure is counteracted by a reflex vasoconstriction and increase in heart rate (Nickerson 1975). Effects associated with both nitrate-induced vasodilation and compensatory vasoconstriction have been reported in studies evaluating the human response to Otto Fuel II and/or propylene glycol dinitrate exposure.

Headaches (of likely vasodilatory origin) were reported as a frequent symptom by workers exposed to Otto Fuel II (ATSDR 1990; Horvath et al. 1981) and by subjects experimentally exposed to propylene glycol dinitrate (Stewart et al. 1974). These reports *are* consistent with the reported ability of aliphatic nitrates to cause meningeal blood vessel dilation which results in a characteristic type of headache (Nickerson 1975). The earliest and most commonly encountered symptom of overexposure to propylene glycol dinitrate (or Otto Fuel II) is headache. In the study by Stewart et al. (1974), the severity and frequency of the headaches decreased with repeated exposures, indicating adaptation to the vasodilatory effects.

In studies of other aliphatic nitrates, continued adaptive vasoconstriction after removal from the source of exposure has been reported to be the cause of coronary insufficiency (Carmichael and Lieben 1963; Morton 1977). The increased incidence of angina pectoris and myocardial infarction among workers exposed to Otto Fuel II (Forman et al. 1987) indicates that compensatory vasoconstriction of the blood vessels of the heart may lead to coronary insufficiency in these workers.

Studies in animals have assessed the vasodilatory activity of propylene glycol dinitrate following exposure by the dermal route (Clark and Litchfield 1969) and following parenteral administration (Air Force 1982b; Clark and Litchfield 1969; Pharmackon 1988). These studies demonstrate that propylene glycol dinitrate causes a decrease in blood pressure that is rapidly corrected. An increase in heart rate also occurs as part of the compensatory response. Comparison of the time-course of the vasodilation with the blood levels of propylene glycol dinitrate and its metabolites indicated that propylene glycol dinitrate levels correlated most closely with the decrease in blood pressure (Clark and Litchfield 1969). Animal studies also suggest that the heart may be adversely affected by exposure to propylene glycol dinitrate. Evidence of myocardial degeneration was observed in rabbits receiving a large dermal dose of propylene glycol dinitrate (Jones et al. 1972).

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These results suggest that persons exposed to sufficient concentrations of Otto Fuel II or propylene glycol dinitrate at hazardous waste sites may experience transitory vasodilation and headaches. The development of a compensatory level of vasoconstriction may also lead to coronary vasospasm in susceptible individuals.

Hematological Effects. In addition to their effects on the cardiovascular system, nitrates are also known to oxidize the heme iron molecule of hemoglobin, resulting in the formation of methemoglobin (Donovan 1990). Experimental acute-duration exposure of humans to low levels of propylene glycol dinitrate did not result in elevation of methemoglobin levels (Stewart et al. 1974). However, studies in animals demonstrated that exposure to high levels of Otto Fuel II or propylene glycol dinitrate for short periods or exposure to low levels for longer periods resulted in elevation of blood methemoglobin levels (Air Force 1982b, 1985a; Cooper et al. 1993; Jones et al. 1972).

Nitrates have also been reported to oxidize the sulfhydryl groups of the globin portion of the hemoglobin molecule resulting in hemoglobin denaturation and hemolysis (ATSDR 1991; Cotran et al. 1989). Evidence of hemolysis and phagocytosis of hemoglobin has been observed in animal studies (Air Force 1985a; Jones et al. 1972). Heavy increases in hemosiderin deposits in the spleen, liver, and kidneys were observed in rats, dogs, and monkeys following continuous inhalation exposure to moderate levels of propylene glycol dinitrate (Jones et al. 1972). Also, decreased red cell count, hemoglobin, and/or hematocrit were observed in rabbits after short-term, high-level dermal exposure (Jones et al. 1972) and dogs after long-term, low-level exposure (Air Force 1985a) to propylene glycol dinitrate.

Comparison of the susceptibility of hemoglobin from various species to oxidation by propylene glycol dinitrate revealed that human hemoglobin is relatively resistant to oxidation (Wyman et al. 1985). Therefore, it is unlikely that exposure to low levels of propylene glycol dinitrate at hazardous waste sites would result in clinically significant methemoglobinemia in most persons. However, some persons are highly susceptible to agents that oxidize hemoglobin (see also Section 2.7), and such persons may experience elevated methemoglobin levels when exposed to Otto Fuel II or propylene glycol dinitrate at hazardous waste sites. Intermediate- and chronic-duration inhalation MRLs were derived based on a study in which dogs exhibited increased methemoglobin levels and decreased red blood cells, hematocrit, and hemoglobin during intermediate-to-chronic inhalation exposures (Air Force 1985a).

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Musculoskeletal Effects. Although no adverse effects on the musculoskeletal system have been identified in humans exposed to Otto Fuel II or its components, a study in rats demonstrated an increase in osteosclerosis in females chronically exposed to propylene glycol dinitrate (Air Force 1985a). No studies were available that corroborated this effect; thus, it is unknown whether it may have been incidental or exposure related. Insufficient information is available to assess the relevance of this finding to persons exposed to Otto Fuel II or propylene glycol dinitrate at hazardous waste sites.

Hepatic Effects. Studies in humans have not demonstrated adverse effects of Otto Fuel II or its components on the liver. However, studies in animals continuously exposed to moderate levels of propylene glycol dinitrate by inhalation (16-35 ppm) have demonstrated degenerative hepatocellular changes (Jones et al. 1972). Intermittent exposures to similar levels did not, however, cause such changes (Air Force 1985a). Thus, it is possible that persons continuously exposed to Otto Fuel II or propylene glycol dinitrate by virtue of their living and/or working in close proximity to a hazardous waste site may also experience some degree of hepatocellular damage.

Renal Effects. No adverse renal effects have been observed in studies of persons exposed to Otto Fuel II or its components. However, a number of animal studies have shown renal toxicity resulting from propylene glycol dinitrate exposure. Evidence of renal toxicity in these studies includes acute tubular necrosis in rats and elevated serum urea nitrogen and decreased serum alkaline phosphatase in monkeys continuously exposed to moderate levels of propylene glycol dinitrate (Jones et al. 1972); vacuolar changes in the proximal convoluted tubules of kidneys of rabbits to which a large dermal dose of propylene glycol dinitrate had been applied (Jones et al. 1972); and decreased urine production and increased red blood cells in the urine of dogs receiving an intravenous dose of 2 mg/kg of propylene glycol dinitrate (Air Force 1982b). The renal toxicity observed in these studies may be the result either one or a combination of the following causes: a direct toxic effect of propylene glycol dinitrate on the kidneys or renal toxicity resulting from the urinary excretion of massive amounts of hemoglobin resulting from hemolysis (Cotran et al. 1989) (see also Hematological Effects above). Based on the observation of renal toxicity in animals, it may be concluded that it is possible that persons exposed to sufficiently high levels of Otto Fuel II or propylene glycol dinitrate at hazardous waste sites may experience renal toxicity.

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Dermal Effects. Animal studies indicate that Otto Fuel II and its components are only very slightly to moderately irritating when applied to rabbit skin (Army 1979; Cooper et al. 1993; Jones et al. 1972; Komarova 1976, 1979; Mallette and Von Haam 1952b), and dibutyl sebacate has been demonstrated to be nonirritating to the skin in humans (Askarova and Muryseva 1975; Mallette and Von Haam 1952b). Thus, it is unlikely that skin contact with Otto Fuel II or its components at hazardous waste sites would result in a severe skin reaction.

Ocular Effects. Slight eye irritation has been reported by subjects experimentally exposed to propylene glycol dinitrate by inhalation (Stewart et al. 1974). This finding has been corroborated in an eye irritation study in which propylene glycol dinitrate was instilled into the eyes of rabbits (Jones et al. 1972). Thus, persons exposed to Otto Fuel II or propylene glycol dinitrate at hazardous waste sites may experience mild eye irritation.

Body Weight Effects. No significant effects on body weight were observed in rats mice, guinea pigs, dogs, and monkeys after intermediate- or chronic-duration exposure to propylene glycol dinitrate (Air Force 1985a; Jones et al. 1972). However, a significant reduction in body weight gain was reported in rats and rabbits exposed to propylene glycol dinitrate dermally during pregnancy (Cooper et al. 1993). The relevance of these results to human health is unknown.

Immunological and Lymphoreticular Effects. Very little information was located regarding immunological effects of Otto Fuel II or its components in humans or animals. Dibutyl sebacate was not shown to cause dermal sensitization when applied to the skin of human volunteers or rabbits (Mallette and Von Haam 1952b). However, 3.3% of workers occupationally exposed to lubricants containing dibutyl sebacate reacted to application of this material in a skin test (Askarova and Muryseva 1975). Hemosiderin deposits were observed in the spleens of dogs and monkeys, but not rats or guinea pigs, exposed continuously for 90 days to atmospheres containing 35 ppm propylene glycol dinitrate (Jones et al. 1972). This response probably reflects increased destruction of erythrocytes. Insufficient information exists to conclude whether exposure to dibutyl sebacate at hazardous waste sites might result in sensitization or whether the other components of Otto Fuel II at hazardous waste sites may cause other adverse immunological effects.

Neurological Effects. Studies in humans have demonstrated adverse effects of acute-duration exposure to low levels of propylene glycol dinitrate (0.5 ppm) on coordination (Stewart et al. 1974)

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and effects of acute-duration exposure to 0.2 ppm on subclinical indicators of neuronal function (Horvath et al. 1981; Stewart et al. 1974). An acute-duration inhalation MRL was derived for propylene glycol dinitrate based on altered visual responses in human subjects voluntarily exposed to propylene glycol dinitrate (Stewart et al. 1974). Animal studies have also demonstrated effects of propylene glycol dinitrate exposure on neuronal function, although most effects (ataxia, decreased consciousness, convulsions) have been observed following inhalation and parenteral exposure to high levels of this chemical (Andersen and Mehl 1979; Bogo et al. 1987; Jones et al. 1972). Behavioral paradigms designed to detect performance decrements caused by low-level exposure to propylene glycol dinitrate have given negative results (Mattsson et al. 1981). Thus, humans may be more sensitive than any of the other species that have been tested to the neurological effects of propylene glycol dinitrate. Based on the effects reported in the studies by Stewart et al. (1974) and Horvath et al. (1981), it is possible that persons exposed to sufficient levels of Otto Fuel II or propylene glycol dinitrate at hazardous waste sites may experience adverse neurological effects.

In vitro studies have demonstrated the ability of propylene glycol dinitrate to disrupt neuromuscular activity (Andersen and Mehl 1979) and to inhibit monoamine oxidase activity (Kalin and Kylin 1969); however, it is unknown what possible role the effects observed *in vitro* might play in the neurotoxicity observed in human subjects.

Headaches have frequently been reported both by workers occupationally exposed to Otto Fuel II and volunteers experimentally exposed to propylene glycol dinitrate (ATSDR 1990; Horvath et al. 1981; Stewart et al. 1974). However, the headaches are most likely associated with meningeal blood vessel dilation (Nickerson 1975) (see also Cardiovascular Effects above).

Reproductive Effects. Studies in humans have begun to address the reproductive toxicity of exposure to Otto Fuel II (Forman 1988). However, for purposes of epidemiological evaluation, the exposed population is still too small to allow meaningful analysis of the data. Animal studies examining reproductive function are limited to a single study that examined the reproductive effects of consumption of large amounts of dibutyl sebacate in rats (Smith 1953) and a dominant lethal study in mice that examined the effects of oral exposure to Otto Fuel II (Navy 1982b). These studies demonstrated no adverse effects of consumption of large amounts of dibutyl sebacate on fertility, litter size, or litter survival or of Otto Fuel II on male fertility. Routine examination of the reproductive organs of animals chronically exposed to propylene glycol dinitrate did not reveal any abnormalities

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(Air Force 1985a); however, the effects of propylene glycol dinitrate and 2-nitrodiphenylamine on reproductive function are unknown. Thus, although dibutyl sebacate does not appear to have any adverse reproductive effects in either sex and Otto Fuel II does not appear to cause adverse effects on male reproductive function, insufficient information exists on propylene glycol dinitrate or 2-nitrodiphenylamine to conclude whether populations exposed to these chemicals or to Otto Fuel II at hazardous waste sites are at risk of significant reproductive toxicity.

Developmental Effects. Developmental toxicity consisting of increased resorptions and decreased fetal viability, weight, and length has been observed following dermal exposure of rats to Otto Fuel II during mid-gestation (Cooper et al. 1993). However, the developmental effects were observed only at maternally toxic doses. Reduced growth of litters of parental rats given very large amounts of dibutyl sebacate in their diets prior to conception has also been reported (Smith 1953). It is unlikely that persons exposed to dibutyl sebacate at hazardous waste sites could be exposed to sufficiently high concentrations of this material to adversely affect development. The absence of information on developmental effects of propylene glycol dinitrate or 2-nitrodiphenylamine precludes estimation of the developmental effects of these components of Otto Fuel II on exposed populations.

Genotoxic Effects. No studies were located regarding the genotoxicity of Otto Fuel II or its components in humans. Two *in vivo* experiments were performed using the oral route of exposure to assess potential genotoxic activity of Otto Fuel II in somatic cells (bone marrow) or germinal cells (dominant lethal mutations in sperm) (Navy 1982b). Slight increases in the frequency of chromosome aberrations were seen in the bone marrow cytogenetic assay, but the finding was not definitive. Similarly, there was no evidence that Otto Fuel II was clastogenic in the dominant lethal assay; however, the number of pregnant females available for analysis did not constitute a reliable sample size. The only *in vivo* assays performed on a fuel component were two assays done with dibutyl sebacate (Wild et al. 1983). Both assays obtained negative results, but were incomplete. *Drosophila melanogaster* allowed to consume a 5% saccharose solution containing 19 mmol/L dibutyl sebacate showed no evidence of sex-linked recessive lethal mutations. Doses of dibutyl sebacate ranging from 943 to 2,829 mg/kg did not increase the frequency of micronuclei induction in bone marrow cells harvested from mice receiving the test compound by intraperitoneal injection (Wild et al. 1983). However, less than the recommended number of animals was used, and there was no indication that the test material was toxic to the animals or cytotoxic to the target organ. Based on the above considerations, no conclusions can be reached from the available *in vivo* genetic toxicology assays; the

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genotoxic activity, if any, of Otto Fuel II and its constituents in whole animals remains uncharacterized.

In vitro genotoxicity studies have been performed with Otto Fuel II and its constituents (propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate). The U.S. Navy (1982b) concluded that Otto Fuel II assayed at toxic levels did not cause a significant increase in the frequency of sister chromatid exchange in mouse lymphoma cells in the presence or absence of rat liver microsomes (S9). There was also no evidence of mitotic delay at any dose. Exposure to severely cytotoxic levels ($\leq 10\%$ survival) of Otto Fuel II in the nonactivated phase of the mouse lymphoma cell forward mutation assay increased the mutation frequencies and the number of mutant colonies. This evidence suggesting mutagenesis was confirmed under S9-activated conditions. Levels ranging from 0.025% to 0.075% with microsomal activation induced significant and dose-related increases in the mutation frequency at the thymidine kinase locus. Therefore, the U.S. Navy (1982b) concluded that Otto Fuel II was mutagenic in this mammalian cell line. Otto Fuel II was also evaluated for mutagenesis in *Saccharomyces cerevisiae* D4 and several histidine-requiring mutant strains of *Salmonella typhimurium* (Navy 1982b). In both of these test systems, Otto Fuel II, both with and without S9 activation, was assayed over an appropriate range of doses (0.005-10 uL/mL) but failed to induce a mutagenic effect.

Several authors (Kleindienst et al. 1985; McGregor et al. 1980; Shepson et al. 1985; Wild et al. 1983) have reported that the components of Otto Fuel II are not mutagenic in microbial systems, but none of their results were conclusive. Propylene glycol dinitrate was negative in the Ames test with or without activation at unspecified concentrations (Kleindienst et al. 1985; Shepson et al. 1985). McGregor et al. (1980) stated that 2-nitrodiphenylamine with or without activation did not cause mitotic recombination in *Saccharomyces cerevisiae*, gene mutation in *S. typhimurium*, or preferential inhibition of a $polA^-$ *Escherichia coli* strain: no data were presented to support their claims. Kononova et al. (1972) concluded that propylene glycol dinitrate produced mutations in the r gene of phage T4B without activation. However, the only dose tested inactivated over 95% of the phage, casting doubts on the validity of the conclusion. Finally, a report that dibutyl sebacate with and without activation was negative in the Ames test presented no study results (Wild et al. 1983). Key *in vivo* genotoxicity studies are presented in Table 2-6 and *in vitro* genotoxicity studies are presented in Table 2-7.

TABLE 2-6. Genotoxicity of Otto Fuel II and Its Components *In Vivo*

Species (test system)	End point	Results	Reference
OTTO FUEL II			
Mice (per os, acute dosing)	Chromosome aberrations	(+) ^a	Navy 1982b
Mice (per os, intermediate dosing)	Chromosome aberrations	- ^a	Navy 1982b
Mice (oral gavage)	Chromosome aberrations in male germ cells	- ^a	Navy 1982b
DIBUTYL SEBACATE			
<i>Drosophila melanogaster</i> (sex linked recessive lethal)	Gene mutation in male germ cells	- ^a	Wild et al. 1983
Mice (intraperitoneal exposure)	Micronuclei formation	- ^a	Wild et al. 1983

^aData presented are insufficient to support authors' conclusions.

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

TABLE 2-7. Genotoxicity of Otto Fuel II and Its Components *In Vitro*

Species (test system)	End point	Results		References
		With activation	Without activation	
OTTO FUEL II				
<i>Salmonella typhimurium</i> (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutation	-	-	Navy 1982b
<i>Saccharomyces cerevisiae</i> (D4)	Gene mutation	-	-	Navy 1982b
Mouse lymphoma (L5187Y cells/TK test)	Forward gene mutation	+	(+)	Navy 1982b
Mouse lymphoma L5178Y cells	Sister chromatid exchange	-	- ^a	Navy 1982b
PROPYLENE GLYCOL DINITRATE				
<i>S. typhimurium</i> TA100	Gene mutation	- ^a	- ^a	Kleindienst et al. 1985
<i>S. typhimurium</i> TA100	Gene mutation	No data	- ^a	Shepson et al. 1985
Bacteriophage T4B (liquid preincubation test)	Gene mutation	No data	+ ^a	Kononova et al. 1972
2-NITRODIPHENYLAMINE				
<i>S. typhimurium</i> (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutation	- ^a	- ^a	McGregor et al. 1980
<i>Escherichia coli</i> (W3110 DNA polymerase I repair test)	Cell death	- ^a	- ^a	McGregor et al. 1980
<i>S. cerevisiae</i> (D5)	Mitotic recombination	- ^a	- ^a	McGregor et al. 1980
DIBUTYL SEBACATE				
<i>S. typhimurium</i> TA100	Gene mutation	- ^a	- ^a	Wild et al. 1983

^a Data presented are insufficient to support authors' conclusions

- = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; TK = thymidine kinase

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Cancer. Studies in humans have not evaluated the carcinogenic potential of Otto Fuel II or its components. Studies in animals have examined the carcinogenic potential of inhalation exposure to propylene glycol dinitrate in rats and mice and have not found strong evidence of a carcinogenic potential (Air Force 1985a). However, the appearance of osteosarcoma and osteoma (rare tumors) in rats, and possibly the increase in the incidence of a common preneoplastic observation (lymphoid hyperplasia) in mice, precludes concluding that exposure to propylene glycol dinitrate is without a carcinogenic risk. No increase in the incidence of neoplasms was noted in a study examining the effects of chronic-duration consumption of large amounts of dibutyl sebacate in rats (Smith 1953); however, the number of rats used in this study was somewhat small for an adequate test of oncogenicity. Thus, insufficient information is available to conclude whether the potential for carcinogenicity exists from exposure to Otto Fuel II or its components at hazardous waste sites.

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 Otto Fuel II and its components are discussed in Section 2.5.1.

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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 Otto Fuel II and its components 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 or Quantify Exposure to Otto Fuel II and Its Components

Extremely limited information was located regarding biomarkers that may be used to estimate exposures to Otto Fuel II. No standard procedures exist for identifying or quantifying exposure to Otto Fuel II. Furthermore, no standard procedures were identified for estimating exposure to the components of Otto Fuel II, propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate. However, based on information on the toxicokinetics of one of the components, propylene glycol dinitrate (Air Force 1982b; Clark and Litchfield 1969; Jones et al. 1972; Mattsson et al. 1981; Stewart et al. 1974), biomarkers for exposure to this component may be proposed.

Propylene glycol dinitrate is rapidly metabolized in the blood (Air Force 1982b; Clark and Litchfield 1969; Kylin et al. 1966). Therefore, measurement of blood levels of this substance or the propylene glycol mononitrates is limited to exposures of a very large magnitude and that occur within a very few hours of the time at which the blood sample is obtained. Also, because propylene glycol dinitrate may be metabolized in isolated blood samples (Clark and Litchfield 1969), steps must be taken to prevent the continued metabolism of this substance. Measurement of propylene glycol dinitrate in the urine or in expired air is of limited value since only a very small fraction of propylene glycol dinitrate is

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excreted by these routes (Air Force 1982b; Clark and Litchfield 1969; Stewart et al. 1974).

Measurement of inorganic nitrate, a metabolic product of propylene glycol dinitrate, in the blood may give some indication of whether exposure levels were high. However, this biomarker may increase following exposure to a large number of other nitrate-containing substances.

Urinary nitrate is the major metabolic marker for propylene glycol dinitrate (Clark and Litchfield 1969). However, measurement of urinary nitrate as a biomarker for exposure to propylene glycol dinitrate is limited by the rapid excretion of nitrate in the urine following exposure (Clark and Litchfield 1969). Within 24 hours of exposure, excretion of nitrate in the urine may be complete. Measurement of urinary nitrate as a biomarker for exposure to propylene glycol dinitrate is also limited by the fact that a large number of inorganic and organic nitrite and nitrate compounds used commercially or therapeutically may produce increased urinary nitrate that is indistinguishable from that caused by propylene glycol dinitrate (Donovan 1990). Such nitrites and nitrates include sodium, potassium, and calcium nitrate (used as fertilizers and food preservatives), bismuth subnitrate (antidiarrheal), ammonium nitrate (diuretic, fertilizer), silver nitrate (topical burn therapy), isosorbide dinitrate or tetranitrate (vasodilator), amyl nitrite (cyanide therapy, vasodilator, abused substance), butyl and isobutyl nitrite (room odorizer, abused inhalant), sodium nitrite (cyanide therapy, food preservative, anticorrosive), and nitroglycerin (vasodilator, explosives).

Methods for detecting 2-nitrodiphenylamine and dibutyl sebacate in body tissues or in excretory products were not located in the literature. In addition, metabolites for these chemicals have not been identified. Thus, measurement of these substances in bodily tissues or excretory products cannot be proposed as useful biomarkers of exposure.

The physiological effects caused by exposure to propylene glycol dinitrate (vasodilation, headache, loss of balance, poor eye-hand coordination, eye irritation, congested nose, nausea, dizziness, and methemoglobin formation) either individually or in combination are not unique to propylene glycol dinitrate exposure. Thus, observation of any one or a combination of these effects does not contribute to the identification or quantification of exposure to propylene glycol dinitrate. For example, eye irritation may be produced by a wide range of substances. Also, a number of other chemicals are known to cause methemoglobinemia (e.g., aniline, benzocaine, chlorate salts, chloroquine, copper sulfate, dapsone, lidocaine, metoclopramide, methylene blue (high doses), monolinuron, naphthalene, nitrates, nitrites, nitrobenzene, nitrogen oxide, nitroglycerin, permanganate salts, phenacetin,

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phenazopyridine, phenols, prilocaine, primaquine, sulfonamides, toluidine) (Donovan 1990). Finally, the combination of symptoms, vasodilation, headache, methemoglobin formation, dizziness, and nausea, may be caused by any of a number of inorganic or organic nitrites or nitrates (Donovan 1990). However, if there is a known exposure event (e.g., accidental release) and a potentially exposed individual experiences vasodilation, headache, eye irritation, nausea, dizziness, and methemoglobinemia, these symptoms may be confirmatory that significant exposure has occurred. It must be mentioned, however, that the absence of headache almost always rules out significant physiological overexposure to propylene glycol dinitrate (or Otto Fuel II) in otherwise healthy individuals.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.5.2 Biomarkers Used to Characterize Effects Caused by Otto Fuel II and Its Components

Very limited information was located regarding effects caused by Otto Fuel II. Also, limited information was located regarding effects caused by two of the components of Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate. The effects caused by propylene glycol dinitrate have, however, been described sufficiently to discuss possible biomarkers for these effects. Such effects include the characteristic type of headache, vasodilation, methemoglobin formation, and dizziness. In experimental exposures in humans, headache due to meningeal blood vessel dilation appears to be the most sensitive effect caused by propylene glycol dinitrate (Stewart et al. 1974). No studies were located that discussed methods for measuring meningeal vessel dilation or the resulting headaches in humans. However, individuals learn to differentiate this type of headache from ordinary headaches. Magnetic resonance imaging and positron emission tomography are noninvasive techniques that may be used to measure cerebral blood flow. Laser-Doppler flowmetry has been used to measure cerebral blood flow; however, in this method, the skull must be opened to allow the probe to be placed in direct contact with the *dura mater* (Dimagl et al. 1989; Saeki et al. 1989).

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Measurement of vasodilation outside of the brain region may be accomplished by monitoring blood pressure (Air Force I982b; Stewart et al. 1974); however, in normal healthy individuals, a rapid vasoconstriction and increase in heart rate may limit the observation of a fall in blood pressure caused by exposure to low concentrations of propylene glycol dinitrate. In fact, in the study by Stewart et al. (1974), a reflex increase in vascular tone may have obscured any fall in blood pressure and may have accounted for the increase in diastolic pressure that was observed.

Experimental exposures in humans indicate that alterations in visual evoked responses may be used to identify subclinical changes in central nervous system activity (Stewart et al. 1974). However, the type of alterations in visual evoked responses produced by propylene glycol dinitrate (increases in the peak-to-peak voltage amplitude) were characterized as being typical of the type of changes produced by central nervous system depressants. Thus, this type of change in the visual evoked responses may be produced by a number of central nervous system depressants. Changes in saccade velocity and delay have been observed in workers acutely exposed to Otto Fuel II in the absence of an effect on coordination or balance (Horvath et al. 1981). The authors of this study suggest that changes in oculomotor responses may be used to detect subclinical changes in central nervous system activity caused by Otto Fuel II. However, similar changes are produced by brain lesions such as those caused by Huntington's chorea, brain stem degeneration, and multiple sclerosis, and from the actions of tranquilizers or alcohol. Thus, these changes are also not specific to the effects of Otto Fuel II. However, in light of a suspected exposure, these tests could be used to assess central nervous system involvement.

A slate-gray cyanosis, appearing first on the mucous membranes, is produced by exposure to relatively high concentrations of propylene glycol dinitrate as a result of the reduced oxygen-carrying capacity of methemoglobin (Donovan 1990; Jones et al. 1972). Cyanosis is not, however, a specific indicator of methemoglobin formation. Cyanosis may also result from increased levels of deoxyhemoglobin in pulmonary or cardiac disease. Cyanosis may also result from agents causing sulfhemoglobin formation. Distinction between cyanosis due to methemoglobinemia and cyanosis due to increased levels of deoxyhemoglobin may be accomplished by shaking a blood sample in the air. In the case of elevated levels of deoxyhemoglobin, the blood should turn bright red, whereas, in the case of methemoglobinemia, the color of the blood (brown or grayish) should be unaffected. Distinction between the cyanosis caused by methemoglobin and that caused by sulfhemoglobin may be achieved by diluting blood in deionized water (1 : 100) with a crystal of potassium cyanide. Blood containing

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methemoglobin should turn pink because of the formation of cyanomethemoglobin. Indicators of methemoglobinemia include a lactate metabolic acidosis and a normal blood oxygen content with low oxygen saturation. Methemoglobin concentrations in the blood may also be quantified spectrophotometrically. These biomarkers are not, however, specific for the methemoglobinemia produced by propylene glycol dinitrate. Heinz bodies in peripheral blood smears may also suggest methemoglobinemia (ATSDR 1991). However, Heinz bodies are not specific for methemoglobinemia.

No information was located regarding possible biomarkers of exposure or effect for 2-nitrodiphenylamine or dibutyl sebacate.

2.6 INTERACTIONS WITH OTHER CHEMICALS

No information was located regarding the influence of other chemicals on the toxicity of Otto Fuel II or its components, propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. Intraperitoneal administration of propylene glycol dinitrate was, however, observed to decrease ethanol consumption in ethanol-addicted rats (Komura 1974). This decrease in ethanol drinking behavior was suggested by Komura (1974) to be related to increased tissue levels of acetaldehyde and ethanol caused by propylene glycol dinitrate-induced depression of alcohol dehydrogenase and acetaldehyde dehydrogenase activities.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to Otto Fuel II and its components than will most persons exposed to the same level of Otto Fuel II and its components in the environment. Reasons include genetic make-up, developmental stage, health and nutritional status, and chemical exposure history. These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

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Review of the literature regarding toxic effects of Otto Fuel II or its components, propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate, did not reveal any populations that are known to be unusually sensitive to these chemicals. However, based on the knowledge of the primary toxic effects of propylene glycol dinitrate, vasodilation and methemoglobin formation, a number of groups may be proposed as being potentially highly sensitive to this chemical.

Groups that may be especially sensitive to the vasodilatory effects of propylene glycol dinitrate include those with arteriosclerotic or cardiac disease (Donovan 1990). In normal individuals, the vasodilation produced by propylene glycol dinitrate is compensated for by a reflex increase in arteriolar constriction and heart rate. Persons with arteriosclerosis have blood vessels with a limited ability to constrict reflexively. Persons with cardiac disease have a limited ability to enhance the mechanical performance of the heart. These groups may experience a greater degree of hypotension than normal individuals exposed to propylene glycol dinitrate.

Individuals with asymptomatic, subclinical coronary heart disease (the majority of the U.S. adult population over age 50) who are chronically exposed to propylene glycol dinitrate vapor concentrations that exceed the industrial time-weighted average-threshold limit value (TWA-TLV) standards, and who develop a tolerance to this exposure, are at risk of experiencing angina pectoris and possible heart injury when the exposure is suddenly terminated, as could occur during a long weekend or vacation away from the job site.

A number of populations may also experience greater sensitivity to the methemoglobinemia produced by propylene glycol dinitrate. For example, some groups may develop greater-than-normal levels of methemoglobin when exposed to propylene glycol dinitrate either as the result of the possession of a type of hemoglobin that is especially easily oxidized or resistant to reduction or as the result of decreased levels of enzymes or cofactors necessary for reduction of methemoglobin. Such persons include infants, persons with the hemoglobin variant, hemoglobin M, and those with congenital deficiencies in NADH-dependent methemoglobin reductase, the enzyme responsible for approximately 95% of the reduction of methemoglobin to hemoglobin. Infants are at increased risk because (1) they possess fetal hemoglobin, which has a greater susceptibility to oxidation than adult hemoglobin, (2) they are deficient in methemoglobin reductase, and (3) they have low levels of erythrocyte NADH (Donovan 1990). Persons with hemoglobin M may have enhanced methemoglobin production because

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this type of hemoglobin is especially easily oxidized and is resistant to the reductive process (Donovan 1990).

The increase in methemoglobin produced by propylene glycol dinitrate may be compounded by the concurrent exposure of some groups to other chemicals that cause methemoglobin formation. For example, munitions workers may also be exposed to the methemoglobin-producing substances: ammonium nitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate because of their use in explosives and pyrotechnics. Also, ingestion of inorganic nitrates (primarily in well water contaminated with nitrogenous fertilizers, animal waste, or seepage from septic tanks) has been shown to cause methemoglobin production. Also, several therapeutic agents such as bismuth subnitrate (antidiarrheal); ammonium nitrate (diuretic); nitroglycerin, isosorbide dinitrate, sodium nitroprusside, pentaerythritol, and erythrityl tetranitrate (vasodilators); metoclopramide (antiemetic); prilocaine, benzocaine, and lidocaine (anesthetics); phenazopyridine (urinary tract analgesic); and dapson, chloroquine, and primaquine (antimalarials) have been shown to increase methemoglobin levels. Abuse of organic nitrites (snappers, poppers) for their enhancement of erections and relaxation of the anal sphincter has also been associated with increased methemoglobin production. Also, accidental or intentional overdose of chlorate salts (found in toothpastes and throat soothants) may cause methemoglobinemia. Finally, exposure to aniline in inks, dyes, shoe polish, paints, varnishes, and gasoline additives has also been reported to induce elevated levels of methemoglobin (Donovan 1990).

The increase in methemoglobin produced by propylene glycol dinitrate may also be compounded by preexisting elevations of methemoglobin due to a medical condition. For example, pregnant women experience an elevation of methemoglobin during pregnancy. The level of methemoglobin peaks at approximately 10.5% at the 30th week of pregnancy and then declines to normal after delivery. Thus, women in the third trimester of pregnancy may be especially sensitive to the increases in methemoglobin produced by propylene glycol dinitrate

Some groups may experience a greater level of tissue anoxia as the result of methemoglobin formation. These include persons with an impaired ability either to oxygenate blood (seen in those with compromised pulmonary function) or to deliver blood to the tissues (seen in those with impaired circulation). In addition, persons suffering from anemia have depressed numbers of red blood cells available to carry oxygen to the tissues (Donovan 1990).

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Finally, some groups are resistant to the methemoglobin antidote, methylene blue (see also Section 2.8). These include persons with congenitally low levels of NADPH-dependent methemoglobin reductase (the enzyme utilized for the reduction of methylene blue) and those with low glucose-6-phosphate dehydrogenase activities (an enzyme necessary for NADPH production in red blood cells) (Donovan 1990).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to Otto Fuel II and its components. 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 Otto Fuel II and its components. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Based on currently available information, the constituent of Otto Fuel II that presents the major health concern is propylene glycol dinitrate. Exposure to propylene glycol dinitrate occurs primarily by inhalation or through dermal absorption. In an acute exposure situation, general recommendations include removing the exposed person from the source of exposure. Dermal absorption may be reduced by removing contaminated clothing, blotting any excess liquid material on the skin with an absorbent material, and washing the skin with copious amounts of water and mild soap (Bronstein and Currance 1988; Donovan 1990; Ellenhom and Barceloux 1988; Stutz and Janusz 1988). If the eyes have been contaminated, they may be flushed with water or normal saline (Bronstein and Currance 1988; Stutz and Janusz 1988). If ingestion of Otto Fuel II or propylene glycol dinitrate has occurred, absorption from the gastrointestinal tract may be limited by administering water or milk for dilution and activated charcoal to adsorb the material (Bronstein and Currance 1988; Donovan 1990; Ellenhom and Barceloux 1988; Stutz and Janusz 1988). The efficacy of gastric decontamination is optimal within 2-4 hours after ingestion (Donovan 1990).

2.8.2 Reducing Body Burden

There are no known specific methods for reducing Otto Fuel II or its components body burden.

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2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The most commonly encountered symptom of overexposure to propylene glycol dinitrate (and Otto Fuel II) is headache due primarily to the vasodilation of cerebral vessels. Controlled human studies have demonstrated that the administration of 100% oxygen by mask, which produces a compensatory constriction of cerebral vessels, is efficacious (Stewart et al. 1974). Strong, black coffee with a high caffeine content, as well as aspirin, have proven helpful in alleviating head pain when there are no contraindications for the administration of either (Stewart et al. 1974).

No agents are known to interfere with the ability of propylene glycol dinitrate to cause vasodilation or cyanosis (resulting from methemoglobin production), but procedures are available and have been recommended to counteract these effects. Hypotension may respond to intravenous fluids and Trendelenburg's position, but vasopressors may be required (Donovan 1990; Ellenhorn and Barceloux 1988). Cyanosis may be treated with high flow (100%) oxygen administration to saturate all remaining normal hemoglobin with, oxygen (ATSDR 1991; Donovan 1990; Ellenhorn and Barceloux 1988). Elevated levels of methemoglobin may be decreased by enhancing the rate of conversion of methemoglobin to hemoglobin. Methylene blue is the antidote of choice in this situation. Ascorbate has been suggested as an alternative reducing agent, but it is believed to have limited efficacy (Donovan 1990; Ellenhorn and Barceloux 1988). Methylene blue is administered intravenously. It is first reduced to leukomethylene blue by NADPH-dependent methemoglobin reductase in the red blood cell. The leukomethylene blue then acts as an electron donor to reduce methemoglobin to hemoglobin nonenzymatically. Use of methylene blue is generally indicated when methemoglobin levels exceed 30% but may be used at lower methemoglobin levels in persons with pulmonary or cardiovascular disease or with preexisting anemia (Donovan 1990; Ellenhorn and Barceloux 1988; Goldfrank et al. 1990). Methylene blue is ineffective in persons with glucose-6-phosphate dehydrogenase deficiency and of limited effectiveness in persons with NADPH-dependent methemoglobin reductase deficiencies (Donovan 1990; Ellenhorn and Barceloux 1988; Goldfrank et al. 1990). Severe hemolytic anemia may develop if given to persons with glucose-6-phosphate dehydrogenase deficiency (ATSDR 1991). Caution should be also used when administering methylene blue to others because high doses (>7 mg/kg) may increase methemoglobin levels and cause hemolysis (Donovan 1990; Ellenhorn and Barceloux 1988). In cases of failure of methylene blue therapy, exchange transfusions have been used to replace hemoglobin and remove the absorbed toxin (Donovan 1990; Ellenhorn and Barceloux 1988).

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Information on the effects of low-level chronic exposure to Otto Fuel II or its components is limited. No treatment strategies were located for minimizing the effects of chronic low-level exposures.

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 Otto Fuel II and its components 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 Otto Fuel II and its components.

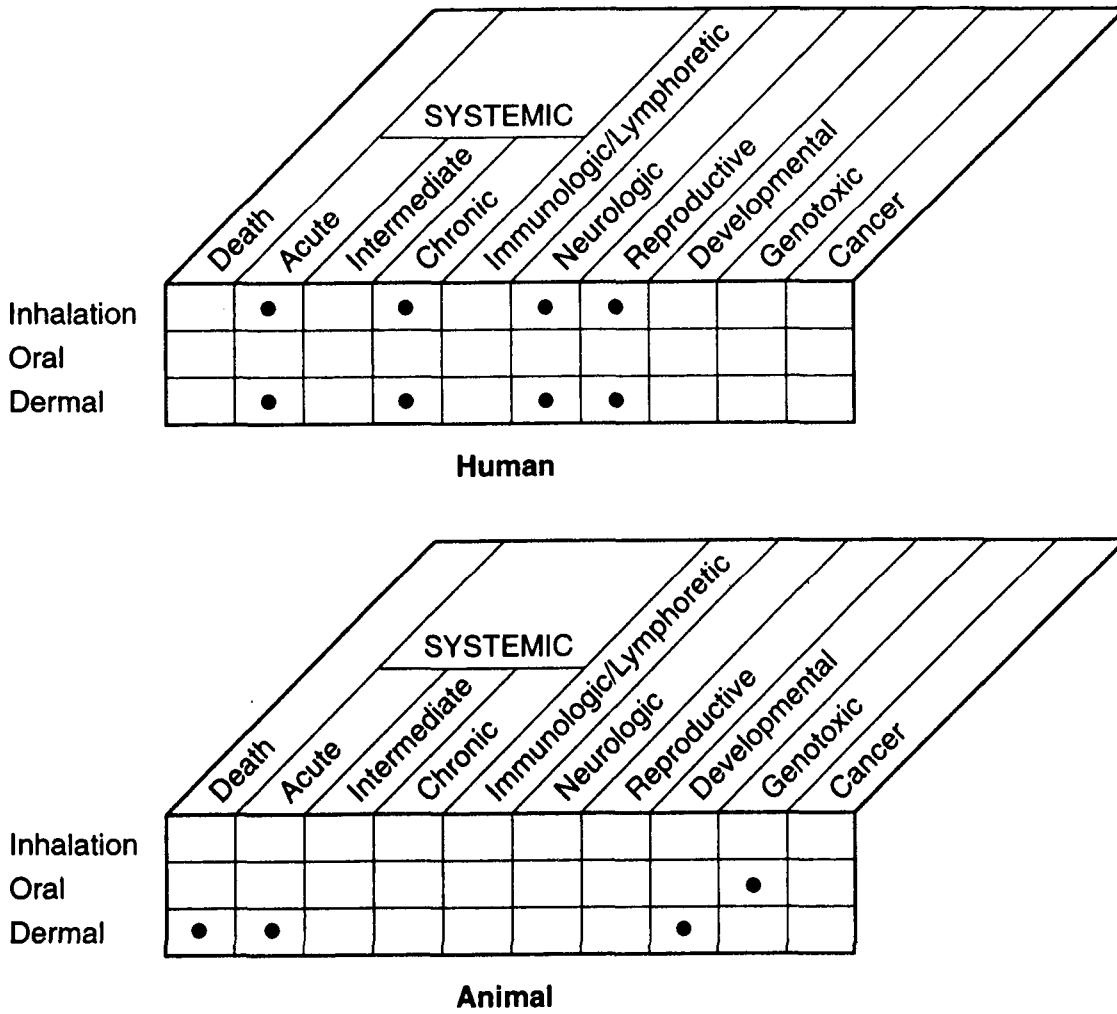
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda may be proposed.

2.9.1 Existing Information on Health Effects of Otto Fuel and Its Components

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to Otto Fuel II and its components are summarized in Figures 2-5 through 2-8. The purpose of these figures is to illustrate the existing information concerning the health effects of Otto Fuel II and its components. 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.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

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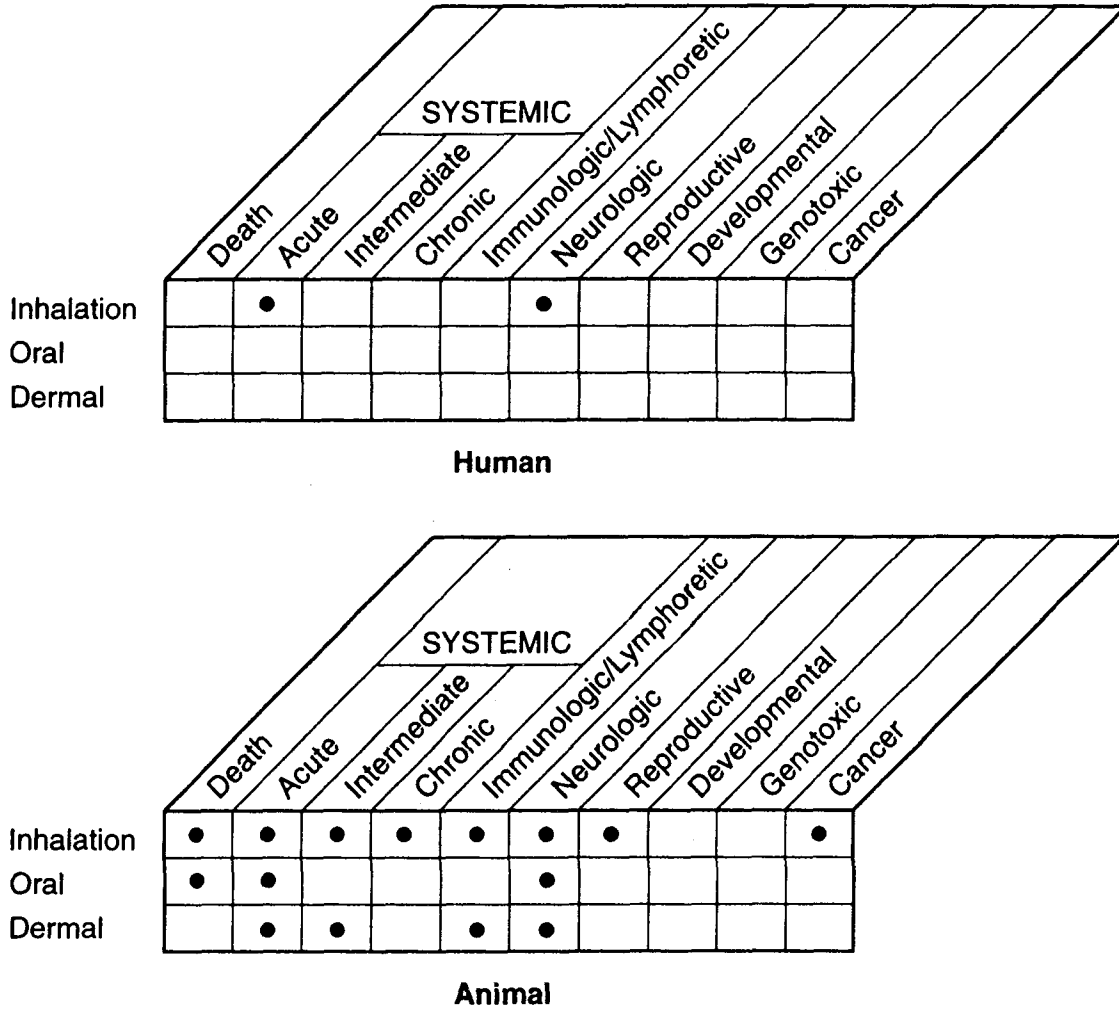
FIGURE 2-5. Existing Information on Health Effects of Otto Fuel II



● Existing Studies

2. HEALTH EFFECTS

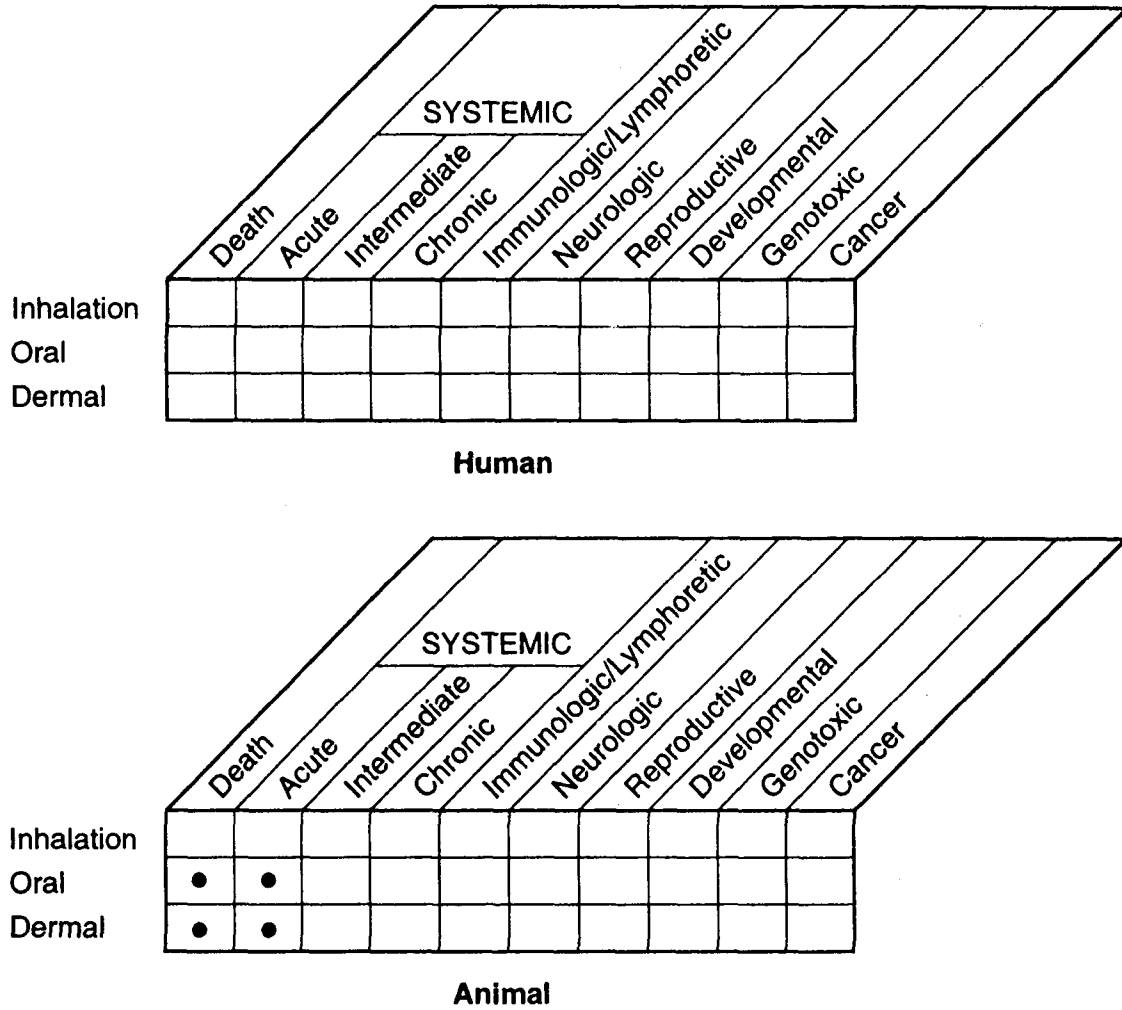
FIGURE 2-6. Existing Information on Health Effects of Propylene Glycol Dinitrate



● Existing Studies

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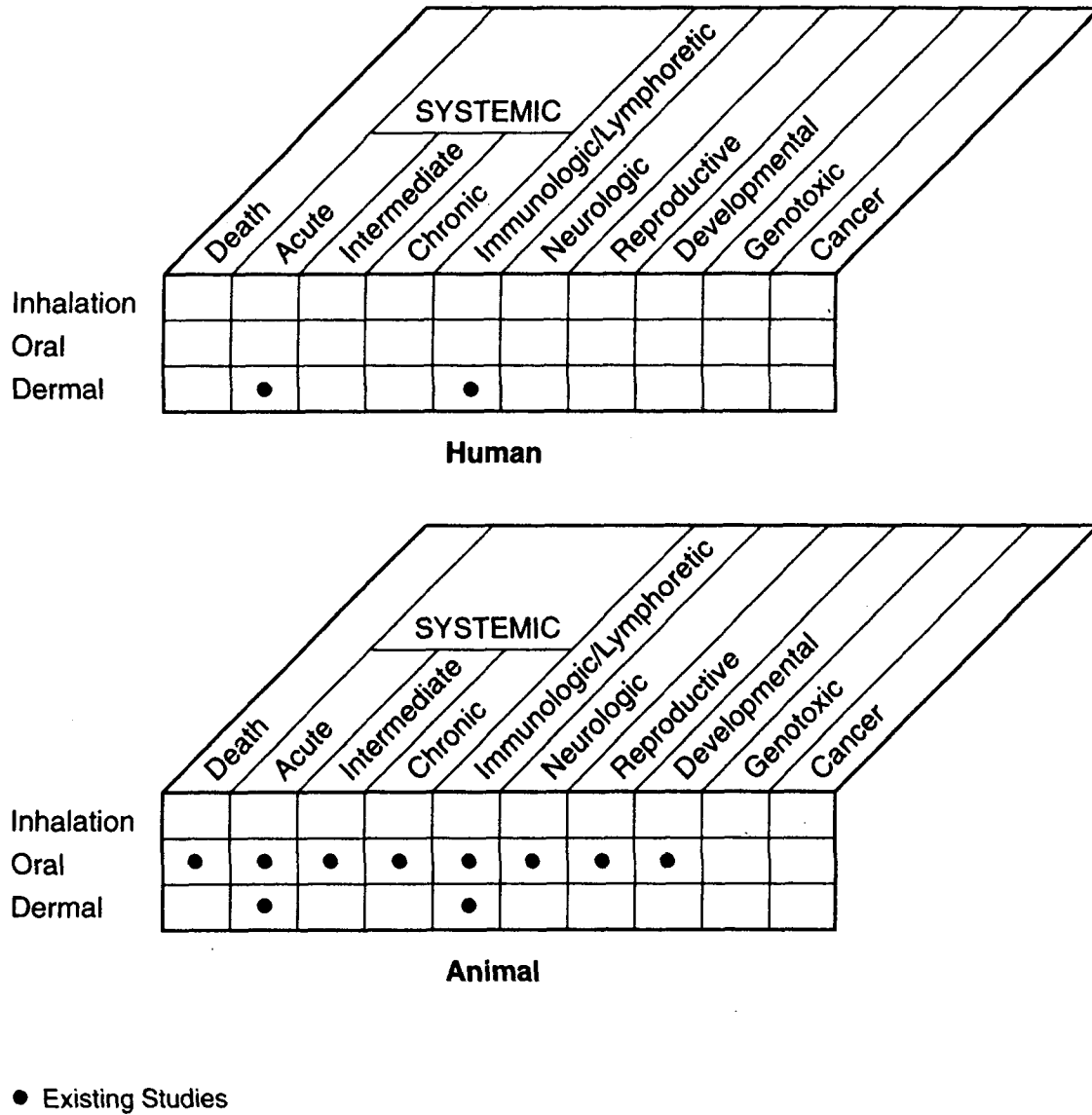
FIGURE 2-7. Existing Information on Health Effects of 2-Nitrodiphenylamine



● Existing Studies

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FIGURE 2-8. Existing Information on Health Effects of Dibutyl Sebacate



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As can be seen in Figures 2-5 through 2-8, the information available on the health effects of Otto Fuel II and its individual components, propylene glycol dinitrate, 2-nitrodiphenylamine, and dibutyl sebacate, varies greatly depending on the specific substance under consideration.

The only information available on the human health effects of Otto Fuel II comes from studies of torpedo maintenance workers and incinerator workers occupationally exposed to Otto Fuel II (ATSDR 1990; Forman 1988; Forman et al. 1987; Horvath et al. 1981). Because occupational exposure could occur either as the result of inhalation of fumes arising from Otto Fuel II or as the result of dermal contact with Otto Fuel II, the information obtained in these studies is indicated as resulting from both inhalation and dermal exposures. One study in rats and rabbits has examined the developmental and maternal toxicity of dermal exposure to Otto Fuel II (Cooper et al. 1993).

The information available on the health effects of propylene glycol dinitrate, the major component of Otto Fuel II, is the most comprehensive of all of the components reviewed. However, the information on this component is not complete. The human data are limited to a study in which volunteers were briefly exposed to propylene glycol dinitrate vapors volatilized from samples of Otto Fuel II (Stewart et al. 1974). The bulk of the animal data on propylene glycol dinitrate is from inhalation studies (Air Force 1985a; Jones et al. 1972; Mattsson et al. 1981). The only animal data resulting from oral exposure are the results of three acute oral toxicity (LD_{50}) studies (Andersen and Mehl 1979; Clark and Litchfield 1969; Jones et al. 1972), and the only dermal data are from two single-dose toxicity studies (Clark and Litchfield 1969; Jones et al. 1972) and two 3-week studies (Andersen and Mehl 1979; Jones et al. 1972).

Very limited information is available on the toxicity of 2-nitrodiphenylamine. The only data located on this component of Otto Fuel II were obtained from a review article (Army 1979) and consisted of a summary of the results of an oral LD_{50} study, a dermal LD_{50} study, and eye and skin irritation studies in animals. (These results are included in the profile because they represent the only information located on 2-nitrodiphenylamine. Attempts are underway to try to obtain the studies upon which the reported results are based.)

The information on the health effects of dibutyl sebacate is somewhat less comprehensive than the information on propylene glycol dinitrate. The human data are limited to two dermal irritation and skin sensitization studies (Askarova and Muryseva 1975; Mallette and Von Haam 1952b). The animal

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data come almost exclusively from oral studies (Komarova 1976, 1979; Smith 1953). The dermal data from animals consist of information obtained in two skin irritation and sensitization studies (Komarova 1976, 1979; Mallette and Von Haam 1952b). Although the information available on dibutyl sebacate is limited, it indicates that this chemical is not very toxic by the oral or dermal routes and not expected to be toxic via other routes.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Populations in areas where Otto Fuel II is disposed of may be exposed to this substance for brief periods. Because of the limited volatility of two of the components of Otto Fuel II, 2-nitrodiphenylamine and dibutyl sebacate, inhalation exposures will be primarily to propylene glycol dinitrate. Insufficient information is available to eliminate any of the components of Otto Fuel II as possible water or soil contaminants, and thus, oral and dermal exposures to Otto Fuel II and its components must be considered. There are acute-duration inhalation exposure data in humans on Otto Fuel II and propylene glycol dinitrate from experimental exposure situations that indicate that the cardiovascular and nervous systems are target organs of these substances by the inhalation route (Horvath et al. 1981; Stewart et al. 1974). Headaches (most likely of cardiovascular origin) and impairment of coordination have been observed (Stewart et al. 1974). An acute-duration inhalation MRL for propylene glycol dinitrate was derived based on the NOAEL for visual evoked responses defined in this study. In addition, subclinical measures of nervous system activity and oculomotor function, have shown effects of low-level inhalation exposure to Otto Fuel II and propylene glycol dinitrate (Horvath et al. 1981; Stewart et al. 1974). No information was available regarding inhalation effects of 2-nitrodiphenylamine or dibutyl sebacate, but the chances for inhalation exposure to these substances is minimal due to their low volatility. No information was available on acute-duration oral exposure of humans to Otto Fuel II or any of its components. Acute-duration dermal exposure of humans to dibutyl sebacate indicated that this substance was not irritating to the skin (Mallette and Von Haam 1952b). Acute-duration occupational exposure to Otto Fuel II, consisting of mixed inhalation and dermal exposures, caused changes in subclinical neurologic parameters (Horvath et al. 1981). However, no information was located on dermal exposure to propylene glycol dinitrate or 2-nitrodiphenylamine.

Acute-duration studies of animals (rats, dogs, and, monkeys) exposed to propylene glycol dinitrate by inhalation support the identification of the neurological and cardiovascular systems as target organs of

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this substance by the inhalation route and indicate that the hematological system is also a target of propylene glycol dinitrate-induced toxicity (Air Force 1985a; Jones et al. 1972; Mattsson et al. 1981). In addition to causing altered neuronal activity and convulsions (Jones et al. 1972; Mattsson et al. 1981) and effects attributable to vasodilation (Jones et al. 1972), increased levels of methemoglobin and decreased hemoglobin and hematocrit were observed after acute-duration inhalation exposure to propylene glycol dinitrate (Air Force 1985a; Jones et al. 1972). Acute-duration studies in rats exposed to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate by the oral route are limited to those with death as the end point (Andersen and Mehl 1979; Army 1979; Clark and Litchfield 1969; Jones et al. 1972; Smith 1953). Because death was the only end point examined in these studies, insufficient information was available to calculate an acute-duration oral MRL. An acute-duration study in rats and rabbits showed that high concentrations of this substance may produce moderate-to-marked skin irritation, methemoglobinemia, and fetal toxicity (Cooper et al. 1993). Acute-duration studies in rats or rabbits exposed to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate by the dermal route indicate that these substances are practically nonirritating to the skin and cause limited eye irritation (Army 1979; Jones et al. 1972; Komarova 1976, 1979; Mallette and Von Haam 1952b). Acute-duration dermal exposure of rats or rabbits to propylene glycol dinitrate also demonstrates the susceptibility of the cardiovascular system to this chemical (Clark and Litchfield 1969; Jones et al. 1972). There are limited pharmacokinetics data regarding propylene glycol dinitrate; however, the data that were obtained do not indicate that route-specific target organs exist. Pharmacokinetics data were extremely limited regarding dibutyl sebacate and 2-nitrodiphenylamine. Thus, it is difficult to speculate on whether similar target organs would be affected across the various potential routes of exposure.

The physical properties of 2-nitrodiphenylamine and dibutyl sebacate do not indicate that inhalation exposure to these substances in the environment is likely. Thus, additional studies examining the effects of acute-duration inhalation exposure to these chemicals are not necessary. Also, lethality studies in rats indicate that oral exposure to 2-nitrodiphenylamine and dibutyl sebacate at levels likely to be encountered in the environment are relatively innocuous. Thus, additional acute-duration studies designed to evaluate the target organs affected by these chemicals do not appear to be necessary. However, additional studies examining the effects of acute-duration oral exposure to propylene glycol dinitrate may be valuable for defining levels of exposure associated with toxicity thresholds. In addition, a study examining the oral and dermal toxicity of the mixture Otto Fuel II would be helpful in eliminating the possibility of synergistic toxic effects of the components. Although the components

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of Otto Fuel II do not appear to cause appreciable dermal or ocular toxicity, additional studies examining the acute-duration dermal toxicity of Otto Fuel II and its components on other target organs would provide valuable information.

Intermediate-Duration Exposure. No studies were located regarding the effects of intermediate-duration exposure to Otto Fuel II or its components in humans by the inhalation, oral, or dermal routes. No studies were located regarding the effects of intermediate-duration exposure to Otto Fuel II in animals by the inhalation, oral, or dermal routes. Intermediate-duration inhalation exposure studies are limited to studies examining the effects of propylene glycol dinitrate in rats, dogs, monkeys, and guinea pigs (Air Force 1985a; Jones et al. 1972; Mattsson et al. 1981). These studies indicate that the blood, liver, and kidneys are target organs of such exposures. In these studies, effects on the blood (elevated methemoglobin and hemolysis of red blood cells) were observed at lower concentrations than effects on the liver or kidneys. An intermediate-duration inhalation MRL was derived for propylene glycol dinitrate based on the observation of elevated methemoglobin and decreased hematocrit, hemoglobin, red blood cells, and reticulocytes in dogs (Air Force 1985a). No intermediate-duration oral studies were located in which animals were exposed to propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate. Therefore, no intermediate-duration oral MRL was derived. Additional animal studies examining the effects of intermediate-duration exposure to dibutyl sebacate do not seem warranted at this time since chronic-duration studies showed the substance to be relatively nontoxic (Smith 1953). However, additional studies examining the effects of intermediate-duration oral exposure to propylene glycol dinitrate, 2-nitrodiphenylamine, and Otto Fuel II would be helpful because of the possibility of oral exposures to these substances. The only intermediate-duration dermal exposure study that was located examined the effects of propylene glycol dinitrate in the rabbit (Jones et al. 1972). This study showed the adverse effects of this chemical on the blood, liver, heart, skin, urinary tract, and possibly the respiratory system. These effects, however, were evident after only a few applications of the chemical; therefore, the study is better categorized as an acute-duration study. Although the effects were produced at relatively high doses, an additional study more clearly delineating the doses associated with the histopathological changes would be helpful. In view of the relatively low chronic-duration toxicity of dibutyl sebacate by the oral route (Smith 1953), additional intermediate-duration studies examining the dermal toxicity of this chemical would not appear to be valuable. However, very little is known regarding the effects of 2-nitrodiphenylamine by the inhalation, oral, and dermal routes of exposure, so studies examining its intermediate-duration toxicity would be helpful.

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Chronic-Duration Exposure and Cancer. Only a few studies have examined the effects of chronic-duration occupational exposure to Otto Fuel II in humans (ATSDR 1990; Forman et al. 1987; Horvath et al. 1981). In these studies, exposures were not quantified and may have been by the inhalation, dermal, or combined inhalation and dermal routes. These studies have examined the effects of such exposures on the likelihood of angina and myocardial infarctions (Forman et al. 1987), the incidence of abortions among exposed female personnel (Forman 1988), and performance on tests of coordination and balance and oculomotor function (Horvath et al. 1981). The study examining myocardial parameters was the only study demonstrating an exposure-related effect (Forman et al. 1987). However, the other two studies were limited by the small numbers of subjects examined, and in the ATSDR study, workers were exposed to other hazardous substances. No studies were located regarding chronic-duration exposures of humans to any of the individual components of Otto Fuel II. Chronic-duration studies in animals are likewise limited in number. The only chronic-duration studies in animals that were located were an inhalation exposure study of the effects of propylene glycol dinitrate in rats, mice, and dogs (Air Force 1985a) and an oral exposure study of the effects of dibutyl sebacate in rats (Smith 1953). Propylene glycol dinitrate exposure resulted in an increase in methemoglobin and decrease in hematocrit, hemoglobin, red blood cells, and reticulocytes in dogs and possibly an increase in mild degeneration of the nasal epithelium of rats at the lowest dose tested (Air Force 1985a). A chronic-duration inhalation MRL for propylene glycol dinitrate was derived from this study based on hematological effects which were observed within several weeks in dogs and continued through 14 months of exposure. No studies were available regarding the effects of chronic-duration oral or dermal exposure to propylene glycol dinitrate in animals. No chronic-duration oral MRL value was derived because of the absence of information on the effects of propylene glycol dinitrate after oral exposure. Studies examining the chronic oral and dermal effects of propylene glycol dinitrate may provide valuable information regarding toxicity thresholds for persons exposed to propylene glycol dinitrate by these routes over an extended period. The absence of toxicokinetic data limits cross-route extrapolation of exposure levels and effects. In addition, a study examining the incidence of anemia or methemoglobin concentration in human populations exposed to propylene glycol dinitrate occupationally may also provide useful information. Long-term consumption of high levels of dibutyl sebacate resulted in no adverse effects in rats (Smith 1953). The absence of toxic effects in animals after oral exposure indicates that chronic dermal exposure studies and epidemiological studies in exposed human populations may not be needed. Based on the absence of information on the effects of chronic-duration oral or dermal exposure to 2-nitrodiphenylamine in humans or animals, studies examining the effects of such exposures in animals would be valuable. Pending the results of the

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animal study, an investigation of the effects of chronic exposure of humans to 2-nitrodiphenylamine may be desirable.

No information was located regarding the carcinogenic potential of Otto Fuel II or its components in humans. The only animal study that examined the oncogenicity of any of the components of Otto Fuel II was a chronic-duration inhalation study of propylene glycol dinitrate exposure in rats and mice (Air Force 1985a). While this study did not demonstrate a marked carcinogenic response in rats or mice to propylene glycol dinitrate, rare tumors (osteoma and osteosarcoma) were observed in the rats, and an increased incidence of a preneoplastic lesion (lymphoid hyperplasia) was observed in the mice. These results are suggestive of a carcinogenic effect, and additional studies examining the carcinogenic potential following inhalation exposure to propylene glycol dinitrate may be necessary to resolve the issue. The duration of the additional studies should be extended to cover the entire lifetime of the species selected, since the Air Force study (1985a) examined the effects of 1-year exposures. True lifetime exposures may reveal more subtle effects. A chronic-duration oral study in rats reported no increase in tumors among animals consuming large amounts of dibutyl sebacate, but the number of animals tested may have been too small to adequately test the carcinogenic potential of this compound. Additional studies examining cancer incidence in human populations chronically exposed to Otto Fuel II or its components and animal studies examining the carcinogenic potential of Otto Fuel II and all three of its components by the oral and dermal routes may provide additional valuable information.

Genotoxicity. Negative results were obtained from well-conducted microbial gene mutation assays (in *Salmonella typhimurium* and *Saccharomyces cerevisiae*) (Navy 1982b). Findings from mammalian cell assays indicated that Otto Fuel II increased the frequency of forward mutations but not sister chromatid exchange in mouse lymphoma cells (Navy 1982b). No *in vitro* cytogenetic studies were found. It is doubtful, however, that a clastogenic response would be uncovered in cultured mammalian cells since Otto Fuel II up to cytotoxic doses did not induce a genotoxic response or cause cell cycle delay on the sister chromatid exchange assay. Because sister chromatid exchange induction frequently occurs at much lower doses than are required to produce chromosome aberrations, it is reasonable to assume that the cytotoxic activity of Otto Fuel II would preclude the assessment of higher concentrations for potential adverse effects on chromosome morphology. Nevertheless, well-conducted *in vivo* studies assessing potential clastogenic activity are needed to complete the genetic toxicology profile for Otto Fuel II. The single positive mammalian cell gene mutation assay should be confirmed either in the same or a different cell line. Since no valid data were found for the constituents of Otto

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Fuel II, a full battery of *in vitro* and *in vivo* tests evaluating the major genetic end points is required. These include gene mutation in microbial and mammalian cells, chromosome aberrations in *in vitro* and *in vivo*, and other mutagenic mechanisms, such as sister chromatid exchange induction in mammalian cells and unscheduled DNA synthesis in primary hepatocytes.

Reproductive Toxicity. One study compared the incidence of spontaneous and induced abortions among female Navy personnel who had occupational exposure to Otto Fuel II with two groups of enlisted females who were not exposed to Otto Fuel II (Forman 1988). Although no increase in the incidence of abortions was observed, the number of women in the exposed group was only five. This severely limits the sensitivity of the comparison. No other studies were located that examined the reproductive effects in humans of exposure to Otto Fuel II or any of its components by the inhalation, oral, or dermal routes of exposure. A single study in laboratory animals has directly examined the reproductive effects of oral exposure to dibutyl sebacate (Smith 1953). This study showed no effect on fertility, litter size, or litter survival of exposed rats. A single dominant lethal study in mice was conducted examining the effects of oral exposure to Otto Fuel II (Navy 1982b); however, no conclusions could be drawn from the fertility phase of the assay because the lower than expected fertility indices in the historic and concurrent negative controls limited the evaluation of reduced fertility in the treatment groups at the majority of mating intervals. No other study has directly examined reproductive function following exposure to Otto Fuel II or any of its components by the inhalation, oral, or dermal route. Routine gross and histopathological examination of reproductive organs (seminal vesicles, prostate, testes, ovaries, uterus, and mammary glands) of mice, rats, and dogs revealed no adverse effects on these tissues following chronic inhalation exposure to propylene glycol dinitrate (Air Force 1985a). Although the oral study using dibutyl sebacate (Smith 1953) indicates that this component of Otto Fuel II has limited reproductive toxicity, other studies examining reproductive function would be helpful. A continuation of the epidemiological study described by Forman (1988) in which the number of exposed female personnel is increased would be useful because this population is expected to have the greatest exposure to Otto Fuel II. In addition, studies in animals examining the reproductive function of inhalation, oral, and dermal exposures to Otto Fuel II or its component propylene glycol dinitrate and of oral and dermal exposure to 2-nitrodiphenylamine are needed to provide information on the possible reproductive effects experienced by persons with these types of exposure to Otto Fuel II at hazardous waste sites.

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Developmental Toxicity. No studies in humans have examined the developmental effects of exposure to Otto Fuel II or any of its components. The only information from studies in animals regarding developmental effects has come from a study examining the developmental toxicity of dermal exposure to Otto Fuel II in rats and rabbits (Cooper et al. 1993) and the reproductive toxicity of oral exposure to dibutyl sebacate in rats (Smith 1953). The dermal Otto Fuel II study suggested that, at maternally toxic doses, developmental toxicity may occur. The study on dibutyl sebacate indicated that this component of Otto Fuel II had only minor adverse effects on the weight gain of offspring of parents that had been fed diets containing dibutyl sebacate. Thus, it is likely that dibutyl sebacate did not account for the developmental toxicity of Otto Fuel II. Studies examining the developmental effects of inhalation and oral exposure of animals to Otto Fuel II, inhalation, oral, and dermal exposure to its component propylene glycol dinitrate, and of oral and dermal exposure of animals to 2-nitrodiphenylamine could provide information on the possible developmental effects experienced by persons with these types of exposure to Otto Fuel II at hazardous waste sites. In addition, as the number of women in the military increases and occupational exposure to Otto Fuel II among females becomes more widespread, studies examining the development of their children could provide information on such effects in a population with known exposure.

Immunotoxicity. Extremely limited information is available on immune function in humans and animals after inhalation, oral, or dermal exposure to Otto Fuel II or any of its components. A skin sensitization, study in humans and rabbits examined the dermal sensitization potential of dibutyl sebacate and found no sensitization 2 weeks following a 2-day dermal exposure (Malette and Von Haam 1952b). In addition, infrequent observation of sensitization to dibutyl sebacate was reported in a study examining the responses of occupationally exposed workers to dibutyl sebacate challenges (Askarova and Muryseva 1975). No other studies examined immune function in humans or animals after inhalation, oral, or dermal exposure to Otto Fuel II or any of its components. Only indirect information regarding the status of the immune system was obtained in animal studies. Results of hematological tests indicated no effect of chronic-duration inhalation or intermediate-duration dermal exposure to propylene glycol dinitrate on total or differential leukocyte counts in rats, dogs, and rabbits (Air Force 1985a; Jones et al. 1972). In addition, the lymph nodes, thymuses and/or spleens of animals in these studies were normal at necropsy. Similarly, no histopathologic alterations were observed in the spleen of rats and guinea pigs in an intermediate-duration inhalation study (Jones et al. 1972) or in the spleen of rats in a chronic-duration oral study (Smith 1953). Because little is known about the effects of Otto Fuel II or its components on the immune system, studies in animals

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examining immune function following inhalation, oral, and dermal exposures to Otto Fuel II or its component propylene glycol dinitrate and following oral or dermal exposure to the components 2-nitrodiphenylamine and dibutyl sebacate would be informative.

Neurotoxicity. Short-term exposure of humans to Otto Fuel II during torpedo maintenance procedures (30-45 minutes per turnaround) and brief experimental inhalation exposures (1-8 hours) to propylene glycol dinitrate at low levels have been shown to cause subclinical alterations in central nervous system activity (Horvath et al. 1981; Stewart et al. 1974). At higher concentrations, tests of coordination were affected, and subjects reported feeling dizzy (Stewart et al. 1974). No studies were located that examined the neurological effects of either 2-nitrodiphenylamine or dibutyl sebacate in humans after exposure by the inhalation, oral, or dermal route. Studies in monkeys have demonstrated central nervous system effects of propylene glycol dinitrate following inhalation exposure only at concentrations higher than those used in the human studies (Jones et al. 1972; Mattsson et al. 1981). In oral and dermal exposure studies, nervous system effects have also been observed in rats and rabbits following administration of large doses of propylene glycol dinitrate (Jones et al. 1972). No information was located on the nervous system effects of 2-nitrodiphenylamine in animals following exposure by the inhalation, oral, or dermal route. The only data located on the possible effects of dibutyl sebacate on the nervous system come from a chronic-duration oral study in rats in which gross and histopathological examination of selected nervous system tissues revealed no abnormalities (Smith 1953). Although humans appear to be more sensitive to the neurological effects of propylene glycol dinitrate than animals, continued studies in animals directed toward elucidating a possible mechanism of action for the effects of propylene glycol dinitrate would be useful. In addition, studies in animals directly examining the effects of 2-nitrodiphenylamine and dibutyl sebacate on neuronal function following oral and dermal exposures could provide information on the potential for effects of these components on the nervous system from exposures at hazardous waste sites.

Epidemiological and Human Dosimetry Studies. Very few epidemiological studies examining the effects of Otto Fuel II were located (Forman 1988; Forman et al. 1987; Horvath et al. 1981). Exposure to Otto Fuel II is known to occur in only a very small segment of the population, namely those persons with exposure to torpedo engines and propellants. Thus, collecting sufficient numbers of exposed persons for meaningful epidemiological studies is difficult. However, because the population most likely to be exposed consists mainly of enlisted personnel, follow-up studies may be more easily performed. Thus, additional studies examining immunological, developmental, reproductive,

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oncogenic, and long-term neurological effects would be valuable if a sufficient number of exposed persons are identified.

Biomarkers of Exposure and Effect.

Exposure. Methods are available for the analysis of propylene glycol dinitrate or its metabolites in expired air, blood, and urine (see Table 6-1). Although measurement of urinary nitrates has been used to indicate exposure to propylene glycol dinitrate, this method is limited by the rapid formation of nitrates in the blood, their rapid excretion, and the large number of other chemicals causing elevated urinary nitrates (Clark and Litchfield 1969; Donovan 1990). A method is available for the analysis of 2-nitrodiphenylamine in hand swabs (Bratin et al. 1981). Procedures for measuring dibutyl sebacate in body tissues or excreta were not found. The rapid hydrolysis of dibutyl sebacate to common organic molecules limits the utilization of measurement of either the parent compound or its metabolites as biomarkers of exposure. Studies delineating the metabolism and excretion of 2-nitrodiphenylamine may suggest potential biomarkers for exposure to this chemical and to Otto Fuel II.

Effect. The effects produced by Otto Fuel II and its components include vasodilation, headache (most likely the result of meningeal vessel dilation), dizziness, and methemoglobin formation. No biomarkers were identified for meningeal vessel dilation or the resulting headaches. Also, monitoring blood pressure as a method for detecting vasodilation may be of limited usefulness because of the ability of normal healthy individuals to compensate for venous dilation with an increase in arteriolar constriction and elevation of heart rate (Air Force 1982b). Methods for spectrophotometrically measuring methemoglobin content of the blood are well defined, and methods for distinguishing cyanosis caused by events other than methemoglobinemia have been identified (Donovan 1990). Neurophysiological parameters such as those assessed by Stewart et al. (1974) and Horvath et al. (1981) are commonly used to monitor for subclinical neurological effects; however, neither of these methods can distinguish propylene glycol dinitrate-induced changes from changes in brain activity due to several other causes. As understanding grows regarding the fundamental mechanism by which Otto Fuel II and propylene glycol dinitrate affect neuronal function, tests may be developed to monitor for the underlying neurological changes caused by these substances.

Absorption, Distribution, Metabolism, and Excretion. The only information regarding the absorption of Otto Fuel II or its individual components in humans comes from an inhalation exposure

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study in which detectable levels of propylene glycol dinitrate were found in the blood of exposed subjects (Stewart et al. 1974). Inhalation exposure of animals to propylene glycol dinitrate has also been demonstrated to result in detectable levels of this substance in the blood (Mattsson et al. 1981). Absorption of all three of the components of Otto Fuel II by the oral route has been indicated by the ability of each of these substances to cause death or systemic toxicity when administered by this route (Andersen and Mehl 1979; Army 1979; Clark and Litchfield 1969; Jones et al. 1972; Smith 1953). Also, dermal absorption of propylene glycol dinitrate has been inferred by the observation of effects on blood pressure after dermal application of this substance (Clark and Litchfield 1969; Godin et al. 1993). True quantitative data on the absorption of propylene glycol dinitrate and dibutyl sebacate following administration by the various routes of exposure may be difficult to obtain because of the rapid metabolism of these substances (Clark and Litchfield 1969; Kylin et al. 1966; Smith 1953). However, information on the relative absorption by the three routes of exposure for propylene glycol dinitrate and by oral and dermal absorption of dibutyl sebacate would be helpful for estimating absorption in humans and clarifying dose limits, and would relate to the practical use of protective clothing. In addition, studies in animals examining absorption of 2-nitrodiphenylamine by the oral and dermal routes of exposure could serve as a basis for estimates of the absorption of this substance by humans.

No information was available on the distribution of 2-nitrodiphenylamine or dibutyl sebacate in either humans or animals following exposure by the inhalation, oral, or dermal route. The only information located on the distribution of propylene glycol dinitrate consists of the observation of this substance in the blood of humans and monkeys that were been exposed by inhalation (Mattsson et al. 1981; Stewart et al. 1974). Therefore, inhalation studies examining the distribution of propylene glycol dinitrate and oral and dermal studies examining the distribution of all three components of Otto Fuel II could provide useful information.

The metabolism of propylene glycol dinitrate and dibutyl sebacate has been studied almost exclusively in *in vitro* studies and/or after parenteral administration (Clark and Litchfield 1969; Godin et al. 1994; Smith 1953). However, the metabolic products of propylene glycol dinitrate have been identified only through the initial steps of metabolism, and the metabolic products of dibutyl sebacate have not been reported. Thus, studies providing the missing information on metabolic products of these chemicals would be useful. No studies were located regarding the metabolism of 2-nitrodiphenylamine. Therefore, studies designed to identify the metabolic products of 2-nitrodiphenylamine would be

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valuable. Also, studies examining the metabolism of the three components of Otto Fuel II after exposure by the three routes might provide information on the effects of the route of exposure on the relative metabolic rates. Studies designed to identify sex- and species-related differences in metabolism would also be valuable.

The excretion of propylene glycol dinitrate in expired air following inhalation exposure has been examined in humans (Stewart et al. 1974). However, outdated methods were used in this study, and no other information on the excretion of propylene glycol dinitrate by humans was located. In addition, urinary excretion of metabolic products of propylene glycol dinitrate after dermal and parenteral administration has been studied in animals (Air Force 1982b; Clark and Litchfield 1969; Jones et al. 1972). However, no study has examined combined expired air, urine, and fecal excretion of propylene glycol dinitrate following exposure by the inhalation, oral, or dermal route. Also, no information exists on excretion of dibutyl sebacate or 2-nitrodiphenylamine from humans or animals after exposure by the inhalation, oral, or dermal route. Thus, studies examining excretion of these substances and their metabolic products following exposure by at least one route would provide useful information on excretory patterns. Studies examining excretion following exposure by more than one route could provide information on possible differences in excretion with varying routes of exposure. Studies on sex- and species-related differences in excretion would also be useful.

Comparative Toxicokinetics. Data are available that suggest that species differences exist with regard to the doses of propylene glycol dinitrate necessary to cause hematological effects (e.g., dogs versus rats) (Air Force 1985a) and neurological effects (e.g., monkeys versus humans) (Mattsson et al. 1981; Stewart et al. 1974). However, the information available on the toxicokinetics of propylene glycol dinitrate in various species is insufficient to speculate on whether the differences are due to variations in toxicokinetic parameters or differences in target organ sensitivities (Wyman et al. 1985). Additional studies examining the absorption, distribution, metabolism, and excretion of propylene glycol dinitrate in the affected species (i.e., following inhalation exposure) may be necessary before such conclusions can be reached.

No data are available on the toxicokinetics of 2-nitrodiphenylamine, and virtually no data are available on the toxicokinetics of dibutyl sebacate. Studies examining the absorption, distribution, metabolism, and excretion of these chemicals in several species are necessary before comparative evaluations based on toxicokinetic differences can be performed.

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Methods for Reducing Toxic Effects. There are good data regarding steps that may be taken to reduce methemoglobin levels and therapeutic measures that may counteract the effects resulting from vasodilation produced by propylene glycol dinitrate. However, additional studies on methods to enhance the breakdown of propylene glycol dinitrate in the blood or to block its effects on vascular smooth muscle would be valuable. No information was located for dealing with toxic effects associated with long-term low-level exposures to Otto Fuel II or its components. Studies examining possible means of preventing coronary vasospasm upon termination of long-term exposures to Otto Fuel II or its major component propylene glycol dinitrate would be helpful. Also, studies assessing appropriate protective equipment (i.e., clothing) and safe methods for handling Otto Fuel II and its components would be helpful.

2.9.3 Ongoing Studies

No ongoing studies evaluating either the health effects or toxicokinetics of propylene glycol dinitrate, 2-nitrodiphenylamine, or dibutyl sebacate, were located. However, ATSDR is conducting a study in Lenoir, North Carolina to evaluate the prevalence of specific diseases and symptoms of a population living within a 15-mile radius of a hazardous waste incinerator where Otto Fuel II was being burned (ATSDR 1992).