2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to isophorone. Its purpose is to present levels of significant exposure for isophorone based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other, interested individuals and groups with (1) an overall perspective of the toxicology of isophorone and (2) a depiction of significant exposure levels associated with various adverse health effects.

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 data in this section are 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, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) 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, determine whether or not the intensity of the effects varies 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 on the tables and graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious effects." Public health officials and project managers concerned with response actions at Superfund 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) are of interest to health professionals and citizens alike.

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For certain chemicals, levels of exposure associated with carcinogenic effects may be indicated in the figures. These levels reflect the actual doses associated with the tumor incidences reported in the studies cited. Because cancer effects could occur at lower exposure levels, the figures also show estimated excess risks, ranging from a risk of one in 10,000 to one in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1980a), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death of humans following inhalation exposure to isophorone.

Acute inhalation exposure of rats, guinea pigs, or mice to isophorone at a concentration of 619 ppm for 6 hours caused slight lacrimation during exposure but did not result in any deaths (Hazleton Labs 1964). Although this study used few animals and did not report the use of controls, the acute exposure level of 619 ppm probably can be considered a NOAEL for lethality because none of the animals of the three species tested died (Table 2-l and Figure 2-l). Hazleton Labs (1965a) statistically determined the 4-hour LC_{50} in rats to be 1238 ppm, with 95% confidence limits of 1008-1531 ppm. The LC_{50} is indicated in Table 2-l and Figure 2-l. Exposure to 885 ppm for 6 hours resulted in the death of l/10 rats (Hazleton Labs 1965a); the exposure of 885 ppm is considered the acute inhalation LOAEL for lethality of isophorone (see Table 2-l and Figure 2-l). The concentration of 885 ppm in air (Hazleton Labs 1965a) is also presented in Table l-2. The cause of death of the rats was not stated, but marked pulmonary congestion was observed. Dutertre-Catella (1976) attempted to determine the LC_{50} of isophorone in rats and rabbits, but saturation of the air at a concentration up to 7000 ppm for 5 hours produced mortality in only 10% of the rats and 30% of the rabbits. The animals became comatose before death and had hemorrhagic lungs, vascular dilation of the alveolar capillaries and peribronchial vessels. Dutertre-Catella (1976) noted that at high concentrations of vapor, which were attained by heating isophorone, an appreciable quantity of the solvent remained suspended as an aerosol in the exposure chamber due to condensation. As the concentrations at which the animals began to die could not be determined from the report, the LOAELs are not known.

TABLE 2-1. Levels of Significant Exposure to Isophorone - Inhalation

Graph Key	Species	Exposure Frequency/ Duration	Effect	NOAEL[b] (ppm)	LOAEL[a] (Effect) Less Serious (ppm)	LOAEL[a] (Effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	rat	once 6 hr		619			Hazleton Labs 1964
2	rat	once 4 hr				1238 (LC_{50})	Hazleton Labs 1965a
3						885 (1/10 died)	
4	mouse	once 6 hr		619			Hazleton Labs 1964
5	guinea pig	once 6 hr		619			Hazleton Labs 1964
Systemic							
6,7	human	15 min	Dermal/ Ocular	10	25 (irritation)		Silverman etal. 1946
8,9	human	7 min	Dermal/ Ocular	18	35 (eye irritation) (throat irritation)		Hazleton Labs 1965b
10	rat	once 6 hr	Respiratory		619 (congestion)		Hazleton Labs 1964
11	mouse	5 min	Respiratory		27.8 (RD_{50})		DeCeaurriz et al. 1981a
12	mouse	once 6 hr	Respiratory		619 (congestion)		Hazleton Labs 1964
Neurological							
13	rat	once 4 hr				1238 (comatose)	Hazleton Labs 1965a
14	mouse	4 hr			89 (behavioral test)		De Ceaurriz et al. 1984
15	mouse	once 4 hr			131 (CNS depression)		De Ceaurriz et al. 1981b

TABLE 2-1 (continued)

Graph Key	Species	Exposure Frequency/ Duration	Effect	NOAEL[b] (ppm)	LOAEL[a] (Effect) Less Serious (ppm)	LOAEL[a] (Effect) Serious (ppm)	Reference
Developmental							
16 17 18	rat	9 d d 6-15gestation 6 hr/d		100		115 (growth retardation) 150 (exencephaly)	Bio/dynamics 1984a,b
19,20	mouse	9 d d 6-15gestation 6 hr/d		115		150 (exencephaly)	Bio/dynamics 1984a,b
INTERMEDIATE EXPOSURE							
Death							
21	rat	4-6 mo 6 hr/d 5 d/wk				500 (1/10 F, 3/10 M died)	Dutertre-Catella 1976
Systemic							
22 23 24	rat	4 wk 6 hr/d 5 d/wk	Hematological Renal Other	37 37	37 (decreased body weight gain)		Hazleton Labs 1968
25 26 27	rat	4-6 mo 6 hr/d 5 d/wk	Respiratory Hepatic Dermal/ Ocular	500 500	500 (ocular and nasal irritation)		Dutertre-Catella 1976
Neurological							
28,29	human	1 mo		1-4	5-8 (fatigue) (malaise)		Ware 1973
CHRONIC EXPSOSURE							
Death							
30	rat	18 mo 6 hr/d 5 d/wk		250			Dutertre-Catella 1976

TABLE 2-1 (continued)

Graph Key	Species	Exposure Frequency/ Duration	Effect	NOAEL[b] (ppm)	LOAEL[a] (Effect) Less Serious (ppm)	LOAEL[a] (Effect) Serious (ppm)	Reference
31	rabbit	18 mo 6 hr/d 5 d/wk		250			Dutertre-Catella 1976
Systemic							
32	rat	18 mo	Respiratory	250			Dutertre-Catella 1976
33		6 hr/d	Hematological	250			
34		5 d/wk	Hepatic		250 (microvacuolization)		
35			Renal	250			
36			Dermal/ Ocular		(irritation of nasal mucosa)		
37	rabbit	18 mo	Respiratory	250			Dutertre-Catella 1976
38		6 hr/d	Hematological	250			
39		5 d/wk	Hepatic		250 (microvacuolization)		
40			Renal	250			
41			Dermal/ Ocular		250 (irritation of nasal mucosa)		

[a]LOAEL - Lowest Observed Adverse Effect Level
[b]NOAEL - No Observed Adverse Effect Level

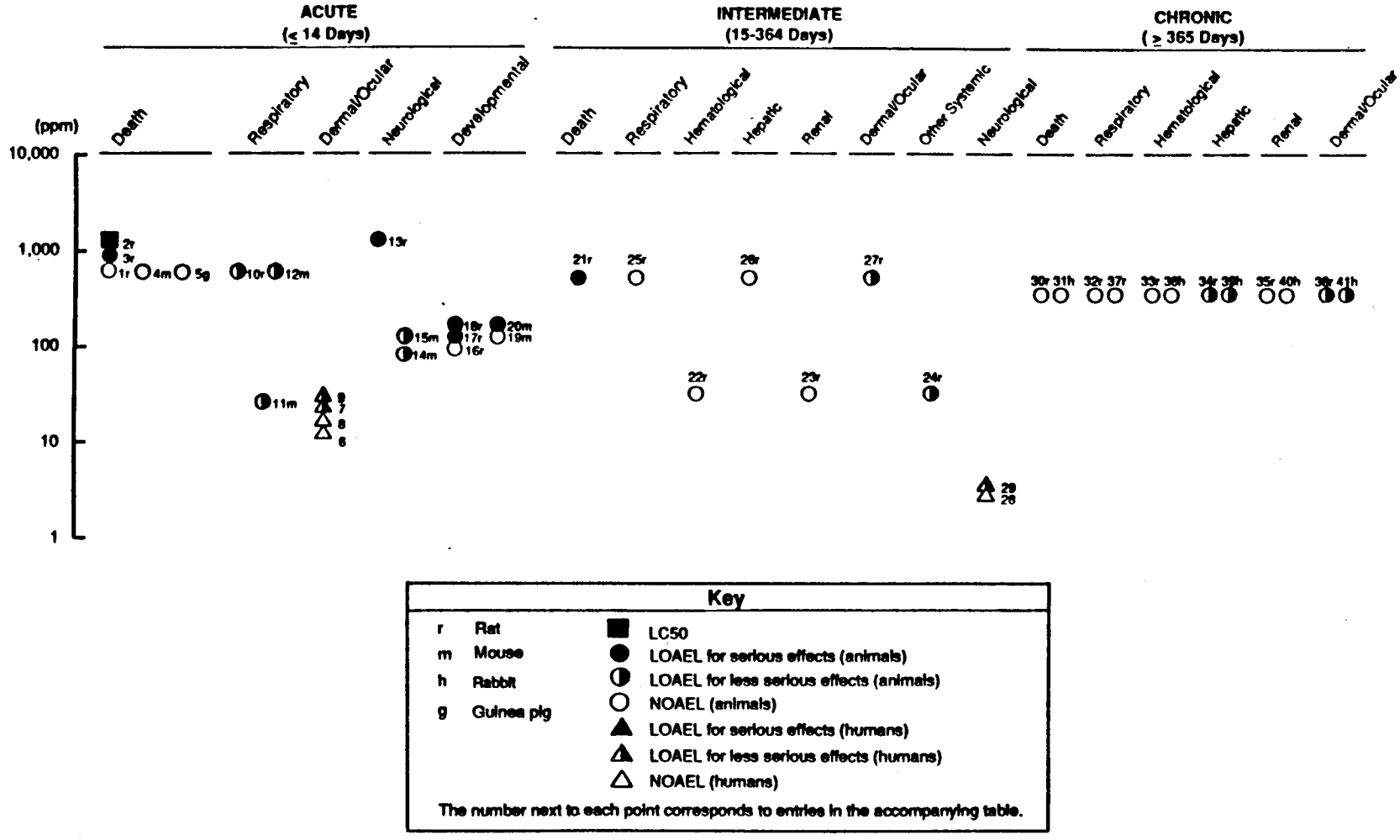

FIGURE 2-1. Levels of Significant Exposure to Isophorone - Inhalation

In an intermediate duration study, l/10 female rats and 3/10 male rats died after being exposed to 500 ppm isophorone for 6 hours/day for up to 6 months (Dutertre-Catella 1976). The 500 ppm concentration is presented in Table 2-l and plotted in Figure 2-l as the intermediate duration LOAEL for lethality. The concentration of 500 ppm (Dutertre-Catella 1976) is also presented in Table l-2. No differences in mortality were observed in rats compared with controls, and no deaths occurred in rabbits exposed to 250 ppm isophorone, 6 hours/day for 18 months (Dutertre-Catella 1976). The 250 ppm concentration is a chronic inhalation NOAEL for lethality in both rats and rabbits (see Table 2-l.and Figure 2-l).

2.2.1.2 Systemic Effects

Respiratory Effects, Lee and Frederick (1981) found that eye, respiratory, and skin irritation were among the complaints of 27/35 workers in a printing plant. Two of the workers (screen printers) were exposed to 8-hour TWA concentrations of isophorone of 0.7 and 14 ppm, but it was not clear whether these two individuals were among those who complained of respiratory irritation. Lee and Frederick (1981) concluded that screen printers are exposed to hazardous concentrations of isophorone and other solvents (xylene, methylene chloride, and toluene).

Very little information is available concerning the systemic effects on the respiratory system of animals due to inhalation exposure to isophorone. Isophorone is irritating to the respiratory tract of animals. DeCeaurriz et al. (1981a) reported that a concentration of 27.8 ppm for 5 minutes caused a 50% decrease (RD_{50}) in the reflex respiratory rate of mice, an indication of respiratory irritation. Because the lowest concentration resulting in a decreased respiratory rate was not indicated clearly in the study, the 27.8 ppm level is indicated as a LOAEL for less serious respiratory effects in mice due to acute inhalation exposure to isophorone (see Table 2-l and Figure 2-l).

Slight lung congestion was observed in rats and mice sacrificed immediately after exposure to 619 ppm isophorone for 6 hours, but not in rats or mice sacrificed 14 days after the exposure (Hazleton Labs 1964), suggesting reversibility of the lesion. This study used only one concentration (619 ppm) and did not report the use of controls. Nevertheless, isophorone is known to be irritating to mucous membranes (see Dermal/Ocular effects below), so it is reasonable to attribute the lung congestion to exposure to isophorone. As the congestion was not a permanent condition, it is not a serious adverse effect; therefore, the 619 ppm level is indicated in Table 2-l and Figure 2-l as a LOAEL for less serious effects on the respiratory system due to acute inhalation exposure to isophorone. Rats and rabbits that were exposed to isophorone at concentrations up to 7000 ppm for 5 hours died and had hemorrhagic lungs with vascular dilation of the alveolar capillaries and peribronchial vessels (Dutertre-Catella 1976). The concentration of 7000 ppm cannot be considered a LOAEL because it was not clear from the report if the animals started dying at

concentrations less than 7000 ppm. Moreover, Dutertre-Catella (1976) noted that at 7000 ppm, a considerable quantity of the isophorone was present in the exposure atmosphere as an aerosol rather than as a vapor. The concentration of 27.8 ppm in air (DeCeaurriz et al. 1981a) was rounded to 28 mm, and the concentration of 619 ppm in air was rounded to 620 ppm (Hazleton Labs 1964) for presentation in Table 1-2.

Severe lung injury consisting of congestion, necrosis, and degeneration was reported in rats and guinea pigs exposed intermittently to 100 ppm, but not to 25 ppm, isophorone for 6 weeks (Smyth et al. 1942). According to Rowe and Wolf (1963), however, later investigation led to the conclusion that the isophorone used by Smyth et al. (1942) contained several highly volatile impurities, a fact unknown to the investigators at the time. These impurities could have contributed to the severity of the observed effects. Moreover, Rowe and Wolf (1963) argued that some of the vapor concentrations reported by Smyth et al. (1942) were higher than could possibly be achieved under the conditions employed and some were overestimated because the vapor concentrations of the impure commercial product within the exposure chamber were determined using an interferometer that had been calibrated against pure isophorone.

These criticisms of the Smyth et al. (1942) study have also been noted by NTP (1986) and ACGIH (1986). Given the uncertainty regarding the results and the exposure levels reported by Smyth et al. (1942), it is inappropriate to consider this study for the determination of levels of significant exposure.

Hazleton Labs (1968) found no treatment-related histopathological lesions in lungs of rats exposed intermittently to 37 ppm for 4 weeks compared with controls. The 37 ppm concentration was the only one tested, and histological examination was limited to 30% of the control and treated rats. Although the limited histological examination could have missed lung lesions in the rats that were not examined (70%), no exposure-related histopathological lesions were observed in the lungs of rats exposed to 500 ppm isophorone for up to 6 months or in rats and rabbits exposed to 250 ppm for 18 months (Dutertre-Catella 1976). The NOAELs for respiratory effects of 500 ppm in rats for intermediate duration exposure and 250 ppm in rats and rabbits for chronic exposure are presented in Table 2-1 and Figure 2-1.

Hematological Effects. No studies were located regarding hematological effects in humans following inhalation exposure to isophorone.

Hematological effects of inhalation exposure of animals to isophorone are not well documented. No studies were located regarding hematological effects in animals following acute inhalation exposure to isophorone. Hazleton Labs (1968) compared the hematological effects of inhalation exposure to isophorone with those of three other ketones in rats. The postexposure values in the treated and control groups were compared with the

pre-exposure values. No extreme variations occurred.in any group, but the isophorone-exposed group (37 ppm for 4 weeks) had an increased percentage of lymphocytes (3% in males, 5.8% in females), decreased percentage of neutrophils (2.8% in males, 5.0% in females), and increased hemoglobin content (1.2-1.4 g/100 ml) compared with the pre-exposure values. Since statistical analysis was not performed, the significance of these findings is not known. The control males also displayed this trend. Because the variations in the isophorone-treated group were slight, and similar to those observed in the unexposed animals, the 37 ppm concentration can be considered a NOAEL for hematological effects of intermediate duration inhalation exposure to isophorone. No exposure-related hematological effects were observed in rats or rabbits exposed to 250 ppm isophorone for 18 months (Dutertre-Catella 1976), which is a NOAEL for chronic exposure. The NOAELs are presented in Table 2-l and Figure 2-l.

Hepatic Effects. No studies were located regarding hepatic effects in humans following inhalation exposure to isophorone.

No studies were located regarding hepatic effects in animals following acute inhalation exposure to isophorone. In the Hazleton Labs (1968) 4-week study, rats exposed to 37 ppm had statistically significant decreased mean absolute liver weights and statistically significant decreased mean liverto-body weight ratios compared with controls. Histological examination of the livers of 30% of the rats revealed no treatment-related liver lesions; therefore, the toxicological significance of the decreased liver weight is probably minimal. As 70% of the rats in each group were not examined histologically, however, the possibility exists that histopathological liver lesions were missed. No exposure-related histopathological liver lesions occurred in rats exposed to 500 ppm isophorone for up to 6 months, but cytoplasmic microvacuolization of hepatocytes was observed in rats and rabbits exposed to 250 ppm isophorone for 18 months (Dutertre-Catella 1976). The NOAEL of 500 ppm for intermediate duration exposure and the LOAEL of 250 ppm for chronic exposure are presented in Table 2-l and Figure 2-l. The concentration of 500 ppm in air (Dutertre-Catella 1976) is also presented in Table l-2.

Renal Effects. No studies were located regarding renal effects in humans following inhalation exposure to isophorone.

No studies were located regarding kidney effects in animals following acute inhalation exposure to isophorone. Smyth et al. (1942) found severe kidney damage, consisting of congestion, necrosis, and degeneration, in rats and guinea pigs exposed intermittently to 100 ppm isophorone for 6 weeks. As noted, however, Rowe and Wolf (1963) criticized this study for using impure isophorone and overestimating the exposure concentrations. Therefore the 100 ppm level cannot be considered the LOAEL for serious effects on the kidney due to inhalation exposure to isophorone.

In the Hazleton Labs (1968) 4-week study, no treatment-related histopathological effects on the kidney were found in rats exposed to 37 ppm. Because the histological examination was performed on only 30% of the treated and control rats, however, the possibility exists that renal lesions were missed. The 37 ppm concentration can be considered an intermediate duration NOAEL for kidney effects, however, because no exposure-related renal effects were detected upon urinalysis and histological examination of rats and rabbits that were exposed to isophorone in air at a concentration of 250 ppm for 18 months (Dutertre-Catella 1976). The NOAELs of 37 ppm for intermediate duration and 250 ppm for chronic exposure are presented in Table 2-l and Figure 2-l.

Dermal/Ocular Effects. Isophorone is irritating to the eyes and mucous membranes of humans. Several studies have attempted to determine the thresholds for eye, nose, and throat irritation for isophorone in humans. As seen from Table 2-1, when the exposure duration was 15 minutes, 10 ppm was tolerated, while 25 ppm produced irritation to the eye, nose, and throat (Silverman et al. 1946). NIOSH (1978a) noted that Silverman et al. (1946) did not discuss acclimatization. In another study, when the exposure duration was 7 minutes, no irritation was reported at 18 ppm, but the threshold for throat irritation was 35 ppm. Eye and nose irritation occurred at 65 ppm, but not at 35 ppm (Hazleton Labs 1965b). Since no exposure concentrations between 35 and 65 ppm were tested, the threshold for eye and nose irritation falls between these concentrations. The subjects were retested after 2 weeks, with no significant difference between the trials. Thus, these thresholds appear to be reliable, although there was some concern that the concentrations were slightly overestimated because the isophorone may not have vaporized completely. Furthermore, only six subjects per group were tested, and there was substantial individual variability in response. Smyth and Seaton (1940) reported that exposure of humans for a few minutes to 40-400 ppm resulted in eye, nose, and throat irritation at all exposures, but Rowe and Wolf (1963) criticized this study for using impure isophorone and overestimating the exposure concentrations. The NOAELs and LOAELs for irritation due to 7 and 15 minutes of exposure in the studies by Silverman et al. (1946) and Hazleton Labs (1965b) are indicated in Table 2-l and Figure 2-l. The concentration of 25 ppm in air (Silverman et al. 1946) is presented in Table l-l.

The irritancy properties of isophorone have also been observed in humans exposed occupationally to isophorone. In an industrial hygiene survey, Kominsky (1981) reported that the eye and nose irritation complained of by a screen printer could'have been caused by 4-minute exposure to 25.7 ppm isophorone, which was measured in the personal breathing zone while the worker was washing a screen. Lee and Frederick (1981) found that eye, respiratory, and skin irritation were among the complaints of 27/35 workers in a printing plant where isophorone and other solvents (xylene, methylene chloride, and toluene) were used. On the day of measurement, two of the screen printers were found to be exposed to 8-hour TWA concentrations of isophorone of 0.7 and 14 ppm, but it was not clear

whether these two individuals were among the workers complaining of irritation. The odor threshold for isophorone in air has been reported to be 0.2 ppm (v/v) (Amoore and Hautala 1983).

Isophorone is also irritating to animals. Smyth et al. (1942) reported conjunctivitis and skin irritation in rats and guinea pigs exposed to isophorone at high concentrations; as discussed above, however, Rowe and Wolf (1963) criticized this study for using impure isophorone and for overestimating the exposure concentrations. As discussed above for respiratory effects, a concentration of 27.8 ppm for 5 minutes caused a 50% decrease (RD_{50}) in the reflex respiratory rate of mice, which indicated sensory irritation rather than a neurological effect (DeCeaurriz et al. 1981a). Irritation of the eyes and nasal mucosa was observed in rats exposed to 500 ppm isophorone in air for up to 6 months and rats and rabbits exposed to 250 ppm for 18 months (Dutertre-Catella 1976). These LOAELs for intermediate and chronic duration exposure are presented in Table 2-l and Figure 2-l. The 250 ppm concentration is also presented in Table 1-2.

Other Systemic Effects. In the 4-week Hazleton Labs (1968) study, exposure of rats to 37 ppm resulted in statistically significant decreased body weight gain. The 37 ppm level can be considered a LOAEL for less serious effects for intermediate inhalation exposure to isophorone (see Table 2-l and Figure 2-l). The concentration of 37 ppm in air is presented in Table l-2.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals following inhalation exposure to isophorone.

2.2.1.4 Neurological Effects

Isophorone affects the central nervous system. In an industrial hygiene survey report, Lee and Frederick (1981) attributed dizziness complained of by workers to exposure to isophorone and other solvents (xylene, toluene, methylene chloride). In a communication to the American Conference of Governmental Industrial Hygienists, Ware (1973) reported that employees exposed for 1 month to 5-8 ppm isophorone complained of fatigue and malaise. Complaints stopped when workroom exposure levels of isophorone were lowered to l-4 ppm. This communication formed the basis for establishing the ACGIH Ceiling Limit of 5 ppm for isophorone (ACGIH 1986). Thus, 5 ppm can be considered a LOAEL and the range of l-4 ppm a NOAEL for neurological effects in humans due to intermediate inhalation exposure to isophorone (see Table 2-l and Figure 2-l). The concentration of 5 ppm in air (Ware 1973) is presented in Table l-l.

Neurological effects of inhalation exposure to isophorone also have been reported in animals. Narcosis and ataxia occurred in rats and guinea pigs at high exposure concentrations for 6-24 hours (Smyth and Seaton 1940),

but Rowe and Wolf (1963) noted that this study used impure isophorone and overestimated the concentrations. DeCeaurriz et al. (1984) found doserelated neurobehavioral effects (decreased immobility in a behavioral despair swimming test) in.mice exposed for 4 hours. The lowest concentration resulting in the behavioral effects was 89 ppm, which is indicated as a less serious LOAEL in Table 2-1 and Figure 2-1. The concentration of 89 ppm in air (DeCeaurriz et al. 1984) is presented in Table l-2.

DeCeaurriz et al. (1981b) also reported that inhalation of isophorone for 4 hours by mice increased the threshold for onset of seizures produced by intravenous administration of pentrazole, indicating that isophorone depressed the central nervous system. The concentration of isophorone that resulted in a 50% increase in the seizure threshold (STI_{50}) was 131 ppm (LOAEL for less serious effects on Table 2-1 and Figure 2-l), with 95% confidence intervals of 113-145 ppm. At the 4-hour LC_{50} of 1238 ppm and higher, rats were ataxic and comatose during exposure, after which they displayed depression and inactivity (Hazleton Labs 1965a). The 1238 ppm concentration is indicated as a LOAEL for serious effects in Table 2-1 and Figure 2-1. These effects were not noted at 885 ppm. Althohgh the exposure concentration of 885 ppm did not result in overt signs of neurotoxicity, more sensitive tests for neurotoxicity (e.g., operant performance, motor activity, electrophysiology), which may have revealed neurobehavioral effects at this level, were not performed, Therefore, the concentration of 885 ppm should not be considered a NOAEL for neurotoxicity for acute inhalation exposure. Rats and rabbits that were exposed to isophorone for 5 hours at concentrations up to 7000 ppm became comatose and died (Dutertre-Catella 1976). The concentration of 7000 ppm cannot be considered a LOAEL because it was not clear from the report whether the animals became comatose at concentrations less than 7000 ppm. Furthermore, at high concentrations, a considerable amount of isophorone was present in the exposure atmosphere as an aerosol rather than as a vapor (Dutertre-Catella 1976).

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans following inhalation exposure to isophorone.

As part of an intermediate duration study, in which rats were exposed to 500 ppm isophorone in air, Dutertre-Catella (1976) mated exposed males with exposed females, control males with exposed females, exposed males with control females, and control males with control females after 3 months of exposure. Exposure of females continued throughout gestation, and they were allowed to deliver. No differences in pregnancy rate or litter size and no abnormalities in pups were found. The pups were not examined for internal malformations; therefore, this study was inadequate to determine developmental effects of isophorone.

In a pilot developmental toxicity study, pregnant rats and mice were exposed by inhalation to isophorone at concentrations up to 150 ppm on days 6-15 of gestation (Bio/dynamics 1984a). Dose-related mild maternal toxicity (increased liver weight and clinical signs) occurred at all concentrations (≥50 ppm) in rats, but there was no clear indication of maternal toxicity in mice. Exencephaly was observed in one late resorption in one litter in the high-concentration group of rats, and in one late resorption in one litter and in two live fetuses in another litter in the high-concentration group of mice. Because this was a pilot study, only 12 female rats and 12 female mice were used, and the fetuses were examined only for gross abnormalities. A second, more complete developmental toxicity study was also performed in two species. Groups of 22 female rats and 22 female mice were exposed on gestation days 6-15 to concentrations of isophorone up to 115 ppm (Bio/dynamics 1984b). In rats, dose-related maternal toxicity (alopecia) was seen at all concentrations (≥25 ppm). In addition, rat dams exposed to 115 ppm had lower body weights than controls on some days. No other indications of maternal toxicity were noted. There was a statistically significant reduction in mean crown-rump length among rat fetuses, but not among litters, in the group exposed to 115 ppm. In mice, the only effect noted was that the mean body weight of dams exposed to 115 ppm isophorone was decreased during one day of the treatment period. Bio/dynamics (1984b) concluded that isophorone was not teratogenic or fetotoxic in rats or mice at concentrations up to 115 ppm. This conclusion is not supported by the results. Although significance across litters was not reached, the reduction in crown-rump length in the offspring of rats exposed to 115 ppm is evidence of growth retardation. Several deviations from the protocol resulted in the failure to perform some of the scheduled fetal examinations. Bio/dynamics (1984b) stated that these deviations did not alter the conclusions. Based on the findings of the second study, Bio/dynamics (1984b) did not regard the occurrences of exencephaly in the pilot study to be treatment-related. This conclusion is untenable because the second study did not test isophorone at 150 ppm, the exposure level at which the exencephaly was observed. Thus, the findings in the pilot study are difficult to interpret; the incidences of exencephaly/litter at the 150 ppm exposure were not significantly different from controls, but this malformation was observed only at the 150 ppm exposure level. Furthermore, the malformation was seen in both species. Although these results are inconclusive, 150 ppm may be a serious LOAEL for malformations in rats and mice, and 115 ppm may be a NOAEL in mice. The concentration of 115 ppm in rats, however, was associated with growth retardation and represents a serious LOAEL for developmental effects of inhalation exposure. The NOAEL for rats is 100 ppm, which was the next lower dose in the Bio/dynamics (1984a,b) studies. The NOAELs and LOAELs are indicated in Table 2-l and Figure 2-l.

2.2.1.6 Reproductive Effects

As discussed above in Section 2.2.1.5, no differences in pregnancy rate or litter size were observed in rats exposed to isophorone in air at 500 ppm

for 3 months before mating (Dutertre-Catella 1976). Since this study did not examine other parameters of reproductive toxicity, 500 ppm cannot be considered a NOAEL for reproductive effects.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following inhalation exposure to isophorone.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals following inhalation exposure to isophorone.

2.2.2 Oral Exposure

No studies were located regarding health effects in humans following oral exposure to isophorone.

2.2.2.1 Death

The oral LD_{50} of isophorone was reported as 3450 mg/kg in male rats (Hazleton Labs 1964) and 2104-2150 mg/kg in female rats (Smyth et al. 1969, 1970). LD_{50} values of 2700 ± 200 mg/kg for male rats, 2100 ± 200 mg/kg for female rats, and 2200 ± 200 mg/kg for male mice also were reported by Dutertre-Catella (1976). The value reported by Hazleton Labs (1964) was estimated because the mortality data did not lend itself to statistical analysis. Furthermore, the doses were widely spaced, and the animals were fasted for only 3-4 hours before dosing, which could have interfered with gastrointestinal absorption of isophorone. Necropsy of rats that died revealed congestion of the lungs, kidneys, adrenals, and pancreas, and gastrointestinal inflammation. Necropsy of rats that survived the 14-day observation period revealed no effects. The studies by Smyth et al. (1969, 1970) were determinations of the joint toxic action of 27 pairs of industrial solvents (see Section 2.7 on Interactions with other chemicals), but the details of the individual LD_{50} determinations and the cause of death were not provided. Nevertheless, the values for isophorone were reproducible in the two studies by Smyth et al. (1969, 1970). The reason for the sex difference is not apparent. The LD_{50}s are indicated in Table 2-2 and Figure 2-2. No short-term studies of isophorone administered in food or drinking water were located. The LD_{50} of -2100 mg/kg, which was determined using liquid isophorone by gavage (Smyth et al. 1969, 1970), was converted to an equivalent concentration of 15,000 ppm in water for presentation in Table l-4.

Lethality data for intermediate duration oral exposure to isophorone are provided for 16 days and for 13 weeks of exposure in the NTP (1986) study. For the 16-day experiment, Table 2-2 and Figure 2-2 indicate 2000 mg/kg/day as a LOAEL for lethality and 1000 mg/kg/day as a NOAEL for

Table 2-2. Levels of Significant Exposure to Isophorone - Oral

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b]	LOAEL[c] (Effect)		Reference
						Less Serious (mg/kg/day)	Serious	
ACUTE EXPOSURE								
Death								
1	rat	(G)	once				2104- 2150 (LD_{50})	Smyth et al. 1969,1970
2,3	rat	(G)	once		1450		3450 (estimated LD_{50})	Hazleton Labs 1964
4	mouse	(G)	once				2200 (LD_{50})	Dutertre-Catella 1976
Neurological								
5,6	rat	(G)	once			1450 (depression)	5000 (prostration)	Hazleton Labs 1964
INTERMEDIATE EXPOSURE								
Death								
7,8	rat	(G)	16 d 5 d/wk (12 doses in 16 d)		1000		2000 (4/5F died) (1/5M died)	NTP 1986
9,10	mouse	(G)	16 d 5 d/wk (12 doses in 16 d)		1000		2000 (100% mort)	NTP 1986
11,12	mouse	(G)	13 wk 5 d/wk		500		1000 (3/10F died)	NTP 1986

Table 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b]	LOAEL[c](Effect) Less Serious (mg/kg/day)	LOAEL[c](Effect) Serious	Reference
Systemic								
13	rat	(F)	90 d	Resp	311.8			AME Inc 1972a
				Cardio	311.8			
				Gastro	311.8			
				Hemato	311.8			
				Musc/Skel	311.8			
				Hepatic	311.8			
				Renal	311.8			
				Derm/Oc	311.8			
				Other	311.8[d]			
14	rat	(G)	13 wk 5 d/wk	Resp	1000			NTP 1986
				Cardio	1000			
				Gastro	1000			
				Hemato	1000			
				Hepatic	1000			
				Renal	1000			
				Derm/Oc	1000			
				Other	1000			
15	mouse	(G)	13 wk 5 d/wk	Resp	1000			NTP 1986
				Cardio	1000			
				Gastro	1000			
				Hemato	1000			
				Hepatic	1000			
				Renal	1000			
				Derm/Oc	1000			
				Other	1000			
16	dog	(C)	90 d	Resp	150			AME Inc 1972b
				Cardio	150			
				Gastro	150			
				Hemato	150			
				Musc/skel	150			
				Hepatic	150			
				Renal	150			
				Derm/Oc	150			
				Other	150			

Table 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b]	LOAEL[c] (Effect) Less Serious (mg/kg/day)	LOAEL[c] (Effect) Serious	Reference
Neurological								
17	rat	(G)	13 wk 5 d/wk			1000[e] (lethargy)		NTP 1986
18	mouse	(G)	16 d 5 d/wk (12 doses 16 d)			1000[e] (stagger)		NTP 1986
CHRONIC EXPOSURE								
Death								
19,20	rat	(G)	103 wk 5 d/wk		250		500 (increased mortality)	NTP 1986
Systemic								
	rat	(G)	103 wk 5 d/wk	Resp	500			NTP 1986
				Cardio	500			
				Gastro	500			
				Hemato	500			
21				Renal		250 (nephropathy)		
				Derm/Oc	500			
22				Other	500			
	mouse	(G)	103 wk 5 d/wk	Resp	500			NTP 1986
				Cardio	500			
23				Gastro		250 (hyperkeratosis)		
				Hemato	500			
24				Hepatic		250[f] (necrosis)		
25				Renal		500 (inflammation)		
26				Other	500			

Table 2-2 (continued)

Graph Key	Species	Route[a]	Exposure Frequency/ Duration	Effect	NOAEL[b]	LOAEL[c](Effect) Less Serious (mg/kg/day)	LOAEL[c](Effect) Serious	Reference
Cancer								
27	rat	(G)	103 wk 5 d/wk				250 (CEL[g]-kidney tumors)-	NTP 1986
28							500 (CEL[g]-preputial gland tumors)	
29	mouse	(G)	103 wk				250 (CEL[g]-	NTP 1986
30			5 d/wk				lymphoma) 500 (CEL[g]-liver, integumentary system tumors)	

[a]G - Gavage, F - feed, C - capsule
[b]NOAEL - No Observed Adverse Effect Level
[c]LOAEL - Lowest Observed Adverse Effect Level
[d]Used to derive intermediate MRL: Uncertainty Factor of 100 (10 for intraspecies variability, 10 for interspecies variability) applied, resulting in a MRL of 3 mg/kg/day.
[e]Plotted under acute duration - see text.
[f]Used to derive chronic MRL: Uncertainty Factor of 1000 (10 for intraspecies variability, 10 for interspecies variability, 10 for use of a LOAEL) applied resulting in a MRL of 0.2 mg/kg/day.
[g]CEL - Cancer Effect Level

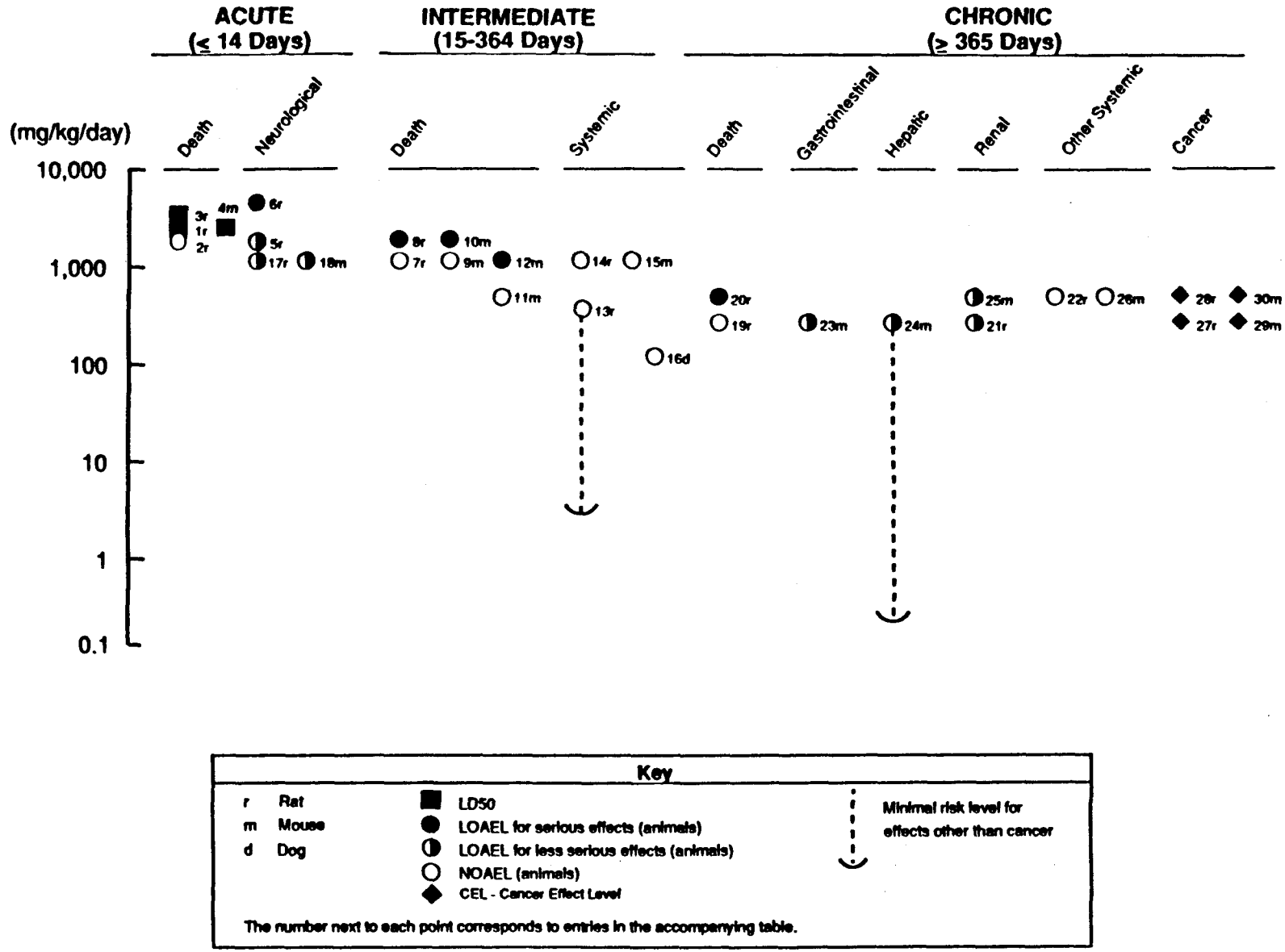

FIGURE 2-2. Levels of Significant Exposure to Isophorone - Oral

lethality in rats and mice. For the 13-week study, ,100O mg/kg/day is the LOAEL for lethality and 500 mg/kg/day is the NOAEL for lethality in mice (see Table 2-2 and Figure 2-2). Although the deaths were considered to be related to isophorone exposure, NTP (1986) did not comment on the cause of death. At 1000 mg/kg/day in the 13-week study, one female rat died during week 5, but NTP (1986) did not comment on whether this death was considered to be related to isophorone exposure.

In the chronic exposure experiment by NTP (1986), male rats treated with 500 mg/kg/day (LOAEL for lethality due to chronic oral exposure on Table 2-2 and Figure 2-2) showed increased mortality, while no increased mortality was observed at 250 mg/kg/day (NOAEL on Table 2-2 and Figure 2-2). NTP (1986) regarded the increased mortality in the male rats to be related to treatment with isophorone, but the cause was not attributed to lesions in any one particular organ system. The increased mortality occurred late in the study (after 96 weeks). No long-term studies of isophorone administered in food or drinking water demonstrating increased mortality were located. The dose levels of 1000 and 2000 mg/kg/day, which were administered to mice by gavage in corn oil for 16 days and 13 weeks (NTP 1986), respectively, were converted to equivalent concentrations of 8000 and 15,000 ppm in food for presentation in Table l-4.

2.2.2.2 Systemic Effects

No reliable studies were located regarding the systemic effects in animals following acute oral exposure to isophorone.

Gastrointestinal Effects. In generally well-conducted, comprehensive go-day studies, no treatment-related grossly or histologically observable lesions were found in the gastrointestinal tract of rats and mice dosed by gavage with isophorone (NTP 1986), rats exposed to isophorone in the diet (AME Inc 1972a), or dogs treated with isophorone in gelatine capsules (AME Inc 1972b). The studies by AME Inc (1972a,b) had several limitations, which include lack of reporting of chemical analysis of feed formulations and statistical methods in the rat study, and failure to examine all animals histologically in both studies. Despite the limitations, the results reported for gastrointestinal effects, as well as for other endpoints, are probably valid because the doses are lower than the NOAELs for the same endpoints in the NTP (1986) go-day (13-week) study. The highest doses administered in these studies were 311.8 mg/kg/day in the diet in rats (AME Inc 1972a), 1000 mg/kg/day by gavage in rats and mice (NTP 1986), and 150 mg/kg/day in dogs (AME Inc 1972b), which are indicated as NOAELs for systemic toxicity due to intermediate oral exposure in Table 2-2 and Figure 2-2.

In the chronic gavage study by NTP (1986), hyperkeratosis of the forestomach was observed in isophorone-treated mice at both doses (Table 2-2 and Figure 2-2), but not in rats. The lowest dose of 250 mg/kg/day is indicated as a LOAEL for less serious gastrointestinal effects due to

chronic oral exposure to isophorone. No long-term studies of isophorone administered in food or drinking water demonstrating gastrointestinal effects were located. The dose level of 250 mg/kg/day, which was administered by gavage in corn oil (NTP 1986), was converted to an equivalent concentration of 1900 ppm in food for presentation in Table l-4.

Hepatic Effects. No significant differences between pre-exposure and post-exposure levels of serum electrolytes, blood glucose and sulfhydride radicals, SGOT, SGPT, serum creatine phosphokinase, or serum lactic dehydrogenase were found in rabbits treated by gavage with isophorone at a dose of 1000 mg/kg/day, 2 days/week for 2 weeks (Dutertre-Catella 1976). No other indices of liver toxicity were examined; therefore, the dose of 1000 mg/kg/day cannot be considered a NOAEL for liver effects. In the go-day studies conducted by NTP (1986) and AME Inc (1972a,b), no treatment-related grossly or histologically observable lesions were found in the livers of rats and mice dosed by gavage with isophorone (NTP 1986), rats treated with isophorone in the diet (AME Inc 1972a), or dogs treated with isophorone in gelatine capsules (AME Inc 1972b). These studies were comprehensive and generally well-conducted. The highest doses administered in these studies were 311.8 mg/kg/day in the diet in rats (AME Inc 1972a), 1000 mg/kg/day by gavage in rats and mice (NTP 1986), and 150 mg/kg/day in dogs (AME Inc 1972b), which are indicated as NOAELs for systemic effects due to intermediate oral exposure in Table 2-2 and Figure 2-2.

No treatment-related gross or histopathological lesions in the liver were observed in rats in the chronic experiment by NTP (1986), but dosed male mice had increased coagulative necrosis and hepatocytomegaly, along with increased incidences of hepatocellular adenomas and carcinomas (see Section 2.2.2.8 on carcinogenicity). Female mice, however, did not have treatment-related lesions in the liver. The highest dose (500 mg/kg/day) is indicated as a NOAEL for liver effects in rats, while the low dose (250 mg/kg/day) is indicated as a LOAEL for non-neoplastic liver lesions in mice due to chronic oral exposure to isophorone (see Table 2-2 and Figure 2-2). No long-term studies of isophorone administered in food or drinking water demonstrating hepatic effects were located. The dose level of 250 mg/kg/day, which was administered.by gavage in corn oil (NTP 1986), was converted to an equivalent concentration of 1900 ppm in food for presentation in Table l-4.

Renal Effects. Gross and histological examination of the kidneys of rats and mice treated with isophorone by gavage (NTP 1986), rats fed diets containing isophorone (AME Inc 1972a), or dogs treated with isophorone in gelatine capsules (AME Inc 1972b) for 90 days revealed no treatmentrelated lesions. In the NTP (1986) go-day studies, recuts and special stains of kidney tissues were performed to confirm the lack of response on the kidney. Thus, the highest doses administered in these studies (311.8 mg/kg/day in the diet in rats, 1000 mg/kg/day by gavage in rats and mice, and 150 mg/kg/day in dogs (AME Inc 1972b) are NOAELs for systemic effects due to intermediate oral exposure (see Table 2-2 and Figure 2-2).

2. HEALTH EFFECTS

The kidney is a target organ for chronic oral exposure to isophorone. In the NTP (1986) study, dosed male mice, but not female mice, had increased incidences of chronic focal inflammation of the kidney, but no other lesions. The 500 mg/kg/day dose is indicated as a LOAEL for less serious renal effects in mice due to chronic oral exposure in Table 2-2 and Figure 2-2. Dosed male rats had increased incidences of tubular cell hyperplasia (possibly pre-neoplastic), epithelial cell hyperplasia of the renal pelvis, and tubular mineralization at both doses. The incidence of tubular mineralization was higher in low dose males than in high dose males, and was coincident with chronic nephropathy, the incidence of which was also higher at the low dose. For this reason, NTP (1986) stated that the nephropathy was probably not the cause of the increased mortality in the high dose males. Male rats also had increased incidences of tubular cell adenomas and carcinomas (see Section 2.2.2.8 on carcinogenicity). As discussed in Section 2.3 (Relevance to Public Health), the tubular cell lesions may be unique to male rats. Dosed female rats had increased incidences of nephropathy, which may have been related to isophorone exposure. The dose of 250 mg/kg/day is indicated as a LOAEL for less serious effects on the kidney in rats for chronic oral exposure to isophorone in Table 2-2 and Figure 2-2. No long-term studies of isophorone administered in food or drinking water demonstrating renal effects were located. The dose level of 250 mg/kg/day, which was administered by gavage in corn oil (NTP 1986), was converted to an equivalent concentration of 5000 ppm in food for presentation in Table 1-4.

Other Systemic Effects. Mean body weight gain was decreased in rats treated by gavage with isophorone at 1000 mg/kg/day, but not at 500 mg/kg/day in a 16-day experiment by NTP (1986). The mice in the 16-day study did not have decreased growth. Body weight changes were not consistent or dose-related in the rats or mice treated with up to 1000 mg/kg/day in the 13-week experiment or up to 500 mg/kg/day in the 103-week experiment (NTP 1986). Male rats treated with 233.8 mg/kg/day had transient decreases in body weight gain during the go-day feeding study by AME Inc (1972a), but body weights were not significantly different from controls at the end of the study. As 1000 mg/kg/day was a NOAEL for body weight changes in rats and mice in 13-week experiments, the decreased mean body weight at the same dose in the 16-day study cannot be considered an adverse effect. Therefore, 1000 mg/kg/day is a NOAEL for body weight changes in rats and mice for intermediate duration exposure.

In the well-conducted, comprehensive go-day studies, no treatmentrelated grossly or histologically observable lesions were found in the lungs, hearts, hematopoietic tissues, or in the skin or eyes of rats and mice dosed by gavage with isophorone (NTP 1986), rats treated with isophorone in the diet (AME Inc 1972a), or dogs treated with isophorone in gelatine capsules (AME Inc 1972b). Furthermore, no changes in hematological indices and no histopathological lesions in the skeletal muscle were found in rats or dogs in the studies by AME Inc (1972a,b). The

highest doses administered in these studies were 311.8 mg/kg/day in the diet in rats (AME Inc 1972a), 1000 mg/kg/day by gavage in rats and mice (NTP 1986), and 150 mg/kg/day in dogs (AME Inc 1972b), which are indicated as NOAELs for systemic effects due to intermediate oral exposure in Table 2-2 and Figure 2-2.

A minimal risk level (MRL) for intermediate oral exposure can be derived from the NOAELs for systemic endpoints. The highest NOAELs for systemic effects of intermediate oral exposure were the 1000 mg/kg/day doses in rats and mice in the 13-week (NTP 1986) study. Increased mortality was observed in mice at 1000 mg/kg/day, however, precluding the use of this dose. Based on the dose of 311.8 mg/kg/day in rats (AME Inc 1972a), an intermediate duration oral MRL of 3 mg/kg/day was calculated, as described in the footnote in Table 2-2. The MRL for intermediate exposure can be compared to existing State and Federal criteria levels (see Chapter 7) or to amounts of the chemical encountered in environmental or occupational situations (see Chapter 5).

Other than the gastrointestinal, hepatic, and renal lesions described above, no treatment-related gross or histopathological lesions were observed in the lungs, heart, hematopoietic organs, skin, or other organs and tissues of rats and mice in the chronic experiment by NTP (1986). There was a dose-related increased incidence of fatty metamorphosis of the adrenal cortex in the male rats, but NTP (1986) stated that the biological significance of this observation is not known. The highest dose (500 mg/kg/day) is indicated as a NOAEL for systemic effects other than gastrointestinal, hepatic, and renal toxicity due to chronic oral exposure to isophorone (Table 2-2 and Figure 2-2). As discussed above under these endpoints, the LOAEL for gastrointestinal, hepatic, and renal effects is 250 mg/kg/day, (as the dose was given 5 days/week, it is equivalent to 179 mg/kg/day). A MRL for chronic oral exposure can be derived from the LOAELs for systemic effects. Based on the dose of 179 mg/kg/day, a chronic oral MRL of 0.2 mg/kg/day was calculated as described in the footnote to Table 2-2. The MRL has been converted to an equivalent concentration in food (7 ppm) for presentation in Table 1-3.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in animals following acute oral exposure to isophorone.

Histological examination of organs and tissues of the immune system did not reveal any effects in rats or mice treated by gavage with isophorone.for 13 or 103 weeks (NTP 1986), in rats treated with isophorone in the diet for 13 weeks (AME Inc 1972a), or in dogs treated with isophorone in gelatine capsules for 13 weeks (AME Inc 1972b). In none of these studies, however, were specific tests of immune function performed. Such tests of immune function are more likely to detect immunological effects than are

histological examinations. Therefore, the doses used in these studies cannot be considered NOAELs for effects on the immune system.

2.2.2.4 Neurological Effects

Neurological effects of isophorone have been observed in animals after oral dosing. In an acute study, rats treated by gavage with isophorone at 5000 mg/kg displayed depression, ptosis, absence of righting reflex, and prostration (LOAEL for serious effects due to acute oral exposure on Table 2-2 and Figure 2-2); 4/5 died within 2 days after dosing (Hazleton Labs 1964). At 1450 mg/kg, depression was observed but the rats recovered within 2 days (LOAEL for less serious effects for acute oral exposure in Table 2-2 and Figure 2-2). No signs of neurotoxicity occurred at 417 mg/kg. In the 16-day NTP (1986) study, mice treated by gavage at 1000 mg/kg/day, but not at 500 mg/kg/day, staggered after dosing, indicating an acute response to the high dose. Similarly, in the 13-week NTP (1986) study, rats given 1000 mg/kg/day, but not 500 mg/kg/day, were sluggish and lethargic after dosing, also indicating an acute response to the high dose. Therefore, 1000 mg/kg is the acute LOAEL for less serious neurological effects (Table 2-2 and Figure 2-2). Although the doses of 417 mg/kg and 500 mg/kg did not result in overt signs of neurotoxicity, more sensitive tests for neurotoxicity (e.g., operant performance, motor activity, electrophysiology), which may have revealed neurobehavioral effects at these doses, were not performed. Therefore, these doses should not be considered NOAELs for neurotoxicity for oral exposure. No short-term studies of isophorone administered in food or drinking water were located. The dose level of 1000 mg/kg in mice, which was administered by gavage in corn oil (NTP 1986), was converted to an equivalent concentration of 8000 ppm in food for presentation in Table l-4.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals following oral exposure to isophorone.

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in animals following acute oral exposure to isophorone.

Histological,examination of reproductive organs did not reveal any effects in rats or mice treated by gavage with isophorone for 13 or 103 weeks (NTP 1986), in rats treated with isophorone in the diet for 13 weeks (AME Inc 1972a), or in dogs treated with isophorone in gelatine capsules for 13 weeks (AME Inc 1972b). In none of these studies, however, were specific tests of reproductive function performed, which would be necessary to rule out an effect. Therefore, the doses used in these studies cannot be considered NOAELs for effects on reproduction.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following oral exposure to isophorone.

2.2.2.8 Cancer

In the chronic gavage study, NTP (1986) concluded that there is "some evidence of carcinogenicity" in male rats due to an increased incidence of relatively rare renal tubular cell adenomas and adenocarcinomas at 250 and 500 mg/kg/day and rare preputial gland carcinomas at 500 mg/kg/day. According to the strict criteria of NTP, "some evidence" in this case means that the study showed a slight increase in uncommon malignant or benign neoplasms. The 250 and 500 mg/kg/day doses are indicated in Table 2-2 and Figure 2-2 as effect levels for carcinogenicity (Cancer Effect Levels, CELs) in rats due to oral exposure to isophorone. Based on the combined incidences of renal tubular cell tumors and preputial gland tumors, EPA (1986, 1987b) proposed an oral q_1* of 4.1×10^{-3} $(mg/kg/day)^{-1}$ for isophorone, but this analysis is under review by the EPA.

In the NTP (1986) study, male mice had marginally increased incidences of hepatocellular tumors and mesenchymal tumors of the integumentary system at 500 mg/kg/day and of malignant lymphomas at 250 mg/kg/day. NTP (1986) considered this evidence to be equivocal because the study showed marginal increases in neoplasms related to isophorone exposure. The doses of 250 and 500 mg/kg/day are indicated in Table 2-2 and Figure 2-2 as CELs for carcinogenicity in mice. There was no evidence of carcinogenicity in female rats or mice.

2.2.3 Dermal/Ocular Exposure

No studies were located regarding health effects in humans following dermal or ocular exposure to isophorone.

2.2.3.1 Death

Hazleton Labs (1964) reported that the dermal LD_{50} of isophorone in rabbits was greater than 3160 mg/kg, the highest dose tested. In this study, the area of application was occluded for 24 hours. Union Carbide (1968), however, reported 1.5 mL/kg (1384 mg/kg) as the dermal LD_{50} in rabbits, but no details of the determination were provided. Therefore, it is not possible to reconcile these contradictory reports, Dutertre-Catella (1976) estimated a dermal LD_{50} of 1200 mg/kg in rabbits. The LD_{50} was difficult to determine with precision because some rabbits died within 6 hours of application and the method requires that the chemical remain on the skin for 24 hours. The rabbits that did not die within 6 hours recovered and were not harmed by doses up to 4000 mg/kg. The dermal LD_{50} is indicated on Table 2-3. When 0.1 or 0.2 mL isophorone was applied to the shaved skin of

Table 2-3. Levels of Significant Exposure to Isophorone - Dermal[a]

	Species	Exposure Frequeny/ Duration	Effect	NOAEL[c]	LOAEL[b](Effect) Less Serious	LOAEL[b](Effect) Serious	Reference
ACUTE EXPOSURE							
Death							
	rabbit	once				1200 mg/kg (LD_{50})	Dutertre-Catella 1976
Systemic							
	guinea pig	once 24 hr	Dermal		dose not specified (irritation)		Eastman Kodak 1967
	rabbit	once	Dermal		0.5 ml (irritation)		Truhaut et al. 1972
	rabbit	once	Ocular			0.02 ml (eye necrosis)	Carpenter and Smyth 1946
	rabbit	30 sec	Ocular			0.1 ml (corneal opacity)	Hazleton Labs 1964
	rabbit	once 24 hr	Dermal	50 mg/kg	200 mg/kg (desquamation)		Hazleton Labs 1964
	rabbit	once 1 or 4 h	Dermal		0.5 ml (irritation)		Potokar et al. 1985
	rabbit	once	Ocular			0.1 ml (eye injury)	Truhaut et al. 1972
Neurological							
	rabbit	once 24 hr		794 mg/kg		3160 mg/kg (CNS depression)	Hazleton Labs 1964

Table 2-3 (continued)

	Species	Exposure Frequeny/ Duration	Effect	NOAEL[c]	LOAEL[b](Effect) Less Serious	LOAEL[b](Effect) Serious	Reference
INTERMEDIATE EXPOSURE							
Death							
	rat	8 wk 7 d/wk				0.1 ml (death of 20% males)	Dutertre-Catella 1976
Systemic							
	rat	8 wk 7 d/wk			0.1 ml (erythema and scar tissue)		Dutertre-Catella 1976

[a]These levels are not displayed graphically because none of the studies used doses expressed in units of $mg/cm^2/day$

[b]LOAEL - Lowest Observed Adverse Effect Level

[c]NOAEL - No Observed Adverse Effect Level

rats for 8 weeks, 20% of the males but none of the females died (Dutertre-Catella 1976) (Table 2-3). No studies were located regarding death of animals following chronic duration exposure to isophorone.

2.2.3.2 Systemic Effects

Dermal/Ocular Effects. Skin irritation was observed in rabbits and guinea pigs following dermal application of isophorone (Eastman Kodak 1967; Hazleton Labs 1964; Potokar et al. 1985; Truhaut et al. 1972). In these studies, undiluted isophorone generally in a volume of 0.5 ml (Table 2-3) was applied to the clipped skin of the animals and held under an occlusive covering. Hazleton Labs (1964) reported doses in units of mg/kg and found that ≥200 mg/kg (LOAEL for less serious effects) resulted in desquamation and erythema, while 50 mg/kg (NOAEL) was without effect. Application of 0.1 or 0.2 mL isophorone to the shaved skin of rats for 8 weeks resulted in erythema and scar tissue formation (Dutertre-Catella 1976). These effects disappeared rapidly after exposure ceased. These doses are indicated in Table 2-3.

Isophorone is also irritating to the eyes of rabbits. Application of 0.02-0.1 ml of undiluted isophorone directly to the eye caused severe injury, cornea1 opacity, and necrosis (Carpenter and Smyth 1946; Hazleton Labs 1964; Truhaut et al. 1972) (see Table 2-3). Hazleton Labs (1964) found that the cornea1 damage was no longer present 14 days after exposure. No studies were located regarding dermal/ocular effects in animals following intermediate or chronic duration exposure to isophorone.

Other Systemic Effects. In rabbits exposed dermally to isophorone at doses up to 3160 mg/kg, no systemic pathological effects were found by gross necropsy (Hazleton Labs 1964), but histological examinations were not performed. In this study, the site of application was occluded for 24 hours to prevent evaporation of isophorone from the skin. No significant differences between pre-exposure and post-exposure levels of serum electrolytes, blood glucose and sulfbydride radicals, SGOT, SGPT, serum creatine phosphokinase, or serum lactic dehydrogenase were found in rabbits treated dermally with 20 mL isophorone (Dutertre-Catella 1976). No other indices of liver toxicity were examined; therefore, the 20 mL dose cannot be considered a NOAEL for liver effects.

2.2.3.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals following dermal or ocular exposure to isophorone.

2.2.3.4 Neurological Effects

In the study by Hazleton Labs (1964), l/4 rabbits exposed dermally to 3160 mg/kg under an occlusive bandage for 24 hours displayed marked depression, labored respiration, sprawling, and depressed reflexes (see

Table 2-3). The other three rabbits at this dosage and at ≤794 mg/kg did not display any signs of toxicity.

No studies were located regarding the following effects in humans or animals following dermal or ocular exposure to isophorone:

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 RELEVANCE TO PUBLIC HEALTH

Death. No information was located regarding death of humans following inhalation, oral, or dermal exposure to isophorone. Concentrations and doses causing death in animals have been reported for acute and intermediate duration inhalation exposures, for acute, intermediate duration, and chronic oral exposures, and for acute and intermediate duration dermal exposure. The acute lethality of isophorone in animals may be due to its effects on the central nervous system. Hazleton Labs (1964) found that rats exposed by inhalation to the LC_{50} were comatose, and rats treated orally with lethal doses displayed depression, absence of righting reflex, and prostration. In the gavage NTP (1986) studies, rats and mice displayed lethargy and staggering after dosing with 1000 mg/kg. The highest dose in the 16-day study (2000 mg/kg/day) was fatal to all the mice and 5/10 of the rats. NTP (1986) did not comment on the cause of death. In the chronic study, high-dose (500 mg/kg/day) male rats had increased mortality (NTP 1986). The increased mortality, which occurred late in the study (after 96 weeks), was considered to be related to isophorone exposure, but NTP (1986) could not relate the cause with effects in any specific organ system. The concentrations or doses of isophorone that would be required to result in the death of humans are not known. As mild neurological effects have been observed in humans exposed to relatively low levels of isophorone, it is likely that if humans were to be exposed to levels high enough to result in death, the cause may be related to more severe CNS effects.

Systemic Effects. The only known effects of isophorone exposure in humans are eye, nose, and throat irritation, and fatigue and malaise. Studies were conducted in humans to determine the thresholds for eye, nose, and throat irritation (25-35 ppm) (Hazleton Labs 1965b; Silverman et al. 1946). The thresholds agree reasonably well with exposure concentrations (≅25 ppm) associated with eye, nose, and skin irritation in occupational settings (Kominsky 1981; Lee and Frederick 1981). The thresholds for irritation are near the OSHA (1989) Permissible Exposure Limit of 25 ppm, and higher than the ACGIH (1988) Ceiling Limit of 5 ppm, which is based on worker complaints of fatigue and malaise (ACGIH 1986).

2. HEALTH EFFECTS

Systemic effects of isophorone observed in animals include pulmonary congestion and hemorrhage, hyperkeratosis of the forestomach, irritation to the skin, eye, and mucous membranes, hematological effects, liver damage, and kidney damage.

The hemorrhaging observed in rats and rabbits (Dutertre-Catella 1976) and the pulmonary congestion observed in rats and mice following acute inhalation exposure to isophorone (Hazleton Labs 1964) is probably related to irritation of mucous membranes. DeCeaurriz et al. (1981a) assessed the sensory irritation of isophorone in mice by measuring the decrease in the respiratory rates, also indicating that isophorone is irritating to the respiratory system. Ocular and nasal irritation also occurred in rats exposed to isophorone in air at 500 ppm for up to 6 months and in rats and rabbits exposed to 250 ppm for 18 months (Dutertre-Catella 1976). Since isophorone is known to be irritating to mucous membranes of humans, inhalation exposure probably could result in pulmonary congestion in h u m a n s . The highest inhalation exposures occur in occupational settings, however, where respiratory irritation has been reported in humans exposed to isophorone and other solvents.

Chronic exposure of mice by gavage to isophorone at 250 mg/kg/day resulted in hyperkeratosis of the forestomach (NTP 1986), an effect that may also be related to the irritating effect on mucous membranes. Although there is no tissue in man that is precisely analogous to the mouse forestomach and the effects of oral exposure of humans to isophorone are not known, ingestion of isophorone could result in gastrointestinal irritation. The minimum concentration of isophorone in water or food necessary to produce the irritation cannot be determined from the available data in animals.

Dermal exposure of rats, rabbits, and guinea pigs results in skin irritation (Dutertre-Catella 1976; Eastman Kodak 1967; Hazleton Labs 1964; Potokar et al. 1985; Truhaut et al 1972). In these studies, liquid isophorone was applied to the skin and the area of application was occluded for 24 hours to prevent evaporation, or to the shaved skin for 8 weeks. While it is unlikely that a human would be exposed in such a manner, screen printers are exposed dermally to both the vapor and the liquid forms of isophorone, which could result in irritation of unprotected skin.

Application of undiluted isophorone to the eyes of rabbits results in severe eye injury (Carpenter and Smyth 1946; Hazleton Labs 1964; Truhaut et al. 1972), which appears to be reversible with time (Hazleton Labs 1964). Isophorone is known to be irritating to the eyes of humans exposed to the vapors (Hazleton Labs 1968; Silverman et al. 1946); it is reasonable to conclude that liquid isophorone splashed directly into the eyes of humans could cause severe eye injury.

When post-exposure values were compared with pre-exposure values, slight changes in the percentage of white blood cells and slightly increased hemoglobin content were observed in rats exposed to isophorone by inhalation (Hazleton Labs 1964), but similar changes also occurred in controls. Therefore, toxicological significance of this finding is minimal and the relevance to humans is not known.

Reported effects on the liver include decreased liver weight in rats exposed subchronically by inhalation (Hazleton Labs 1968), cytoplasmic microvacuolization in rats exposed chronically by inhalation (Dutertre-Catella 1976), and increased incidence of coagulative necrosis and hepatocytomegaly in male mice exposed chronically by gavage at 250 and 500 mg/kg/day (NTP 1986). The decreased liver weight in rats exposed by inhalation is of questionable toxicological significance because histological examination of a limited number of rats in the study did not reveal treatment-related liver lesions. The toxicological significance of the microvacuolization observed in liver cells of rats and rabbits is also unknown. The liver lesions observed in male mice in the oral study were not observed in female mice, but the reason for the sex difference is not known. The mechanism by which isophorone produces liver lesions in male mice is not known, but liver lesions are common in aged mice; isophorone may enhance an age-related process. It is not known whether isophorone causes liver effects or enhances age-related processes in humans.

Subchronic inhalation studies of isophorone (Smyth and Seaton 1940; Smyth et al. 1942) reported severe kidney damage in rats and guinea pigs, but these studies have been criticized for using impure isophorone and for overestimating the exposure concentration (Rowe and Wolf 1963). Because of these reports, however, NTP (1986) examined the kidneys twice and used special staining techniques to confirm the lack'of histopathological lesions and protein droplets in the kidneys of rats and mice exposed subchronically to isophorone by gavage. Although the NTP (1986) study did not detect protein droplet formation in the kidneys of rats or mice treated with isophorone (Bucher 1988), protein droplets were found in the kidneys of male rats exposed by inhalation to dihydroisophorone (Hazleton Labs 1968), a metabolite of isophorone. Furthermore, Strasser (1988) found that isophorone and its metabolites, dihydroisophorone and isophorol, induced significant protein droplet formation in the kidneys of male rats treated acutely by gavage. It was not clear if the response was dose-related.

Isolation and analysis of $_{\text{alpha }2}\mu$-globulin from the kidney cytosol of rats treated with isophorone or dihydroisophorone positively identified isophorone or dihydroisophorone, respectively, in the $_{\text{alpha }2}\mu$--globulin samples, Following treatment with isophorol, samples, isophorone was found in the $_{\text{alpha }2}\mu$-globulin indicating that isophorol was metabolized to isophorone. The results of Strasser (1988) are preliminary and require confirmation, but the data suggest that isophorone and its metabolites bind to $_{\text{alpha }2}\mu$--globulin and induce protein droplet nephropathy in male rats.

2. HEALTH EFFECTS

$_{alpha\ 2}\mu$-Globulin is a low molecular weight protein synthesized in large quantities in the male rat liver, secreted into the blood under the influence of testosterone (Alden 1986), and filtered through the glomerulus. The $_{alpha\ 2}\mu$-globulin is reabsorbed by the tubule cells and sequestered into lysosomes, where it is catabolized into amino acids and peptides. In the normal rat kidney, the rate of catabolism of $_{alpha\ 2}\mu$-globulin is relatively slow compared with that of other proteins (Swenberg et al. 1989). In the male F344 rat, protein droplet nephropathy is characterized by accumulation of $_{alpha\ 2}\mu$-globulin in lysosomes, degeneration and necrosis of tubular cells, formation of granular casts, and regeneration (proliferation) of the tubular epithelium (Swenberg et al. 1989). Chemicals that are known to induce protein droplet nephropathy bind to $_{alpha\ 2}\mu$-globulin, yielding a complex that may be more resistant to the proteolytic enzymes in the lysosomes, which leads to the accumulation of the complex in the tubule cells.

$_{alpha\ 2}\mu$-Globulin has not been found in immature male rats, female rats, or humans (Alden 1986). In adult male rats, the protein may function as a pheromone carrier (Alden 1986). If isophorone induces nephropathy by the suggested mechanism, the absence of $_{alpha\ 2}\mu$-globulin in humans raises the question of the relevance to humans of the isophorone-induced kidney lesions in male rats. $_{alpha\ 2}\mu$-Globulin is related to other low molecular weight transport proteins that have been detected in humans (Swenberg et al. 1989), but it is not known whether chemicals that are nephrotoxic to the rat will bind to the human proteins or produce similar effects in humans.

In the chronic gavage study by NTP (1986), dosed male rats had increased incidences of renal tubular cell hyperplasia, epithelial cell hyperplasia of the renal pelvis, and tubular mineralization. The male rats also had increased incidences of renal tubular cell tumors. The hyperplasia of the tubular cells, therefore, may represent a preneoplastic response (see discussion of cancer below). These proliferative kidney lesions were not observed in male or female mice or in female rats. The mechanism for the induction of proliferative kidney lesions may also be related to $_{alpha\ 2}\mu$-globulin-induced nephropathy (see discussion of cancer below), again raising the question of the relevance of the proliferative kidney lesions in male rats to humans. This issue is presently the subject of scientific investigation.

Female rats had increased incidences of age-related nephropathy (NTP 1986). The tubular mineralization in the male rats was coincident with age-related nephropathies, which were more severe in the low-dose males. It is possible, therefore, that isophorone treatment enhanced the age-related nephropathies commonly seen in rats, but it is not known if isophorone could enhance age-related processes in humans.

Neurological Effects. Humans occupationally exposed to isophorone at levels as low as 5-8 ppm have complained of fatigue and malaise (Ware 1973). When workroom levels were lowered to l-4 ppm, complaints ceased. Neurological effects of oral or dermal exposure of humans to isophorone are

not known. Acute exposure of animals to high inhalation concentrations and oral and dermal doses affects the central nervous system as evidenced by such effects as narcosis, staggering, depression, ataxia, lethargy, and prostration and coma. No histologically detectable lesions have been found. in the brain, sciatic nerve, or spinal cord of animals exposed to isophorone by any route for any duration. Although not precisely known, the mechanism by which isophorone induces its neurological effects may involve interference with neuronal impulse transmissions via physical interaction of isophorone with nerve membrane components, as is seen with many organic solvents. The effects in animals at high exposures and the malaise and fatigue in humans at relatively low workroom concentrations of isophorone predict that exposure of humans to high air concentrations or high oral doses could result in severe central nervous system depression.

Developmental Effects. Isophorone has been tested by inhalation for developmental effects in rats and mice. Evidence for intrauterine growth retardation was seen at an exposure concentration of 115 ppm (Bio/dynamics 1984a,b). At 150 ppm, exencephaly was seen in several fetuses. While the incidence of this malformation was not statistically significant, it was seen in both species and only in treated animals. Dose-related maternal toxicity was evident in all treatment groups. Isophorone has not been tested for developmental effects by the oral or dermal route. No studies were located demonstrating that isophorone crosses the placenta in animals or in humans, but there is no reason to assume that it does not do so. It is not known whether isophorone could cause developmental effects in humans.

Genotoxic Effects. No studies were located regarding the genotoxicity of isophorone in humans or animals by the inhalation, oral, or dermal routes. Isophorone has been tested for genotoxic effects in vitro and in mice treated intraperitoneally (Table 2-4). Isophorone was negative for reverse mutations with and without metabolic activation with S9 prepared from rat or hamster livers (NTP 1986). CMA (1984) reported that isophorone was negative both with and without metabolic activation in mouse lymphoma cells, while NTP (1986) found a weakly positive effect without activation in the same cell system. The concentrations of isophorone used by NTP (1986) were at least 60 times higher than those used by CMA (1984). Negative results were found for unscheduled DNA synthesis in rat hepatocytes, for chromosome aberrations in Chinese hamster ovary cells, and in the micronucleus test in mice (CMA 1984; NTP 1986). NTP (1986) found a positive response for sister chromatid exchange in Chinese hamster ovary cells in the absence, but not in the presence, of a metabolic activating system.

In assessing the potential for a chemical to produce heritable mutations in humans, it is necessary to examine the weight of evidence obtained from in vitro tests for mutations in microorganisms and cultured mammalian cells, from in vivo tests of mutations in animals, and from in vitro and in vivo tests for chromosome aberrations in mammalian cells. The strongest evidence would come from the demonstration that a chemical causes mutations or chromosome aberrations in human cells. As no studies were

TABLE 2-4. Genotoxicity of Isophorone In Vitro and In Vivo

End Point	Species (test system)	Result: Without activation	Result: With activation	References
Reverse mutation	Salmonella typhimurium	-	-	NTP 1986
Forward mutation	L5178Y/TK+/- mouse lymphoma cell	+	NT[a]	NTP 1986
		-	-	CMA 1984; McKee et al. 1987
UDS[b]	Rat primary hepatocyte	-	NA[c]	CMA 1984; McKee et al. 1987
SCE[d]	Chinese hamster ovary cells	+	-	NTP 1986
Chromosome aberrations	Chinese hamster ovary cells	-	-	NTP 1986
Micronucleus test	Mouse erythrocytes (mice treated i.p.)	-	NA[c]	CMA 1984; McKee et al. 1987

[a]Not tested.
[b]Unscheduled DNA synthesis.
[c]Not applicable.
[d]Sister chromatid exchange.

located that tested isophorone in cultured human cells or examined the cells of people with known exposure, this evidence is lacking. Of the five experiments that tested whether isophorone caused mutations or chromosome aberration in cultured mammalian cells, only two were positive: a weak mutagenic response in mouse lymphoma cells and a positive test for sister chromatid exchange in Chinese hamster ovary cells in the absence, but not in the presence, of metabolic activation were obtained by NTP (1986). Isophorone was not mutagenic in the Salmonella/microsome assay (NTP 1986). The only in vivo test was the micronucleus test in mice, which was negative (CMA 1984). Therefore, isophorone may be weakly genotoxic in mammalian cells, but the evidence is insufficient to predict that isophorone poses a genotoxic threat to humans.

Cancer. Increased incidences of relatively rare renal tubular cell adenomas and carcinomas were observed in male rats, but the increases were not statistically significant by the Fisher Exact test or the Cochran-Armitage test (NTP 1986). When adjusted for mortality, however, the increased incidences were significantly different from control in the highdose males when analyzed by the Lifetable test and significant for dose-related trend by the Lifetable and the Incidental Tumor tests. As the kidney tumors were not fatal, the appropriateness of Lifetable test for the analysis of these tumors is questionable. The overall unadjusted incidences were significantly different from the historical control incidence by the Fisher Exact test. The kidney tumors were not observed in female rats or in male or female mice.

Isophorone is one of several diverse chemicals that have been found to induce kidney tubular cell tumors in male rats, but not in female rats, male or female mice, hamsters, guinea pigs, dogs, and nonhuman primates (Alden 1986; Swenberg et al. 1989). These chemicals include 1,4-dichlorobenzene, dimethymethylphosphonate, JP-5 jet fuel; d-limonene, pentachloroethane, tetrachloroethylene, and unleaded gasoline, which have also been found to cause protein droplet nephropathy in male rats. As discussed above for systemic renal effects, binding of these chemicals to $_{\text{alpha }2}\mu$-globulin, a protein that appears to be unique to male rats, is believed to be involved in the protein droplet nephropathy. Based on tumor initiation-promotion studies of trimethylpentane, an $_{\text{alpha }2}\mu$-globulin binding component of unleaded gasoline, and the body of data on $_{\text{alpha }2}\mu$-globulin-induced protein droplet nephropathy, the following mechanism has been proposed for the formation of tumors in the male rat kidney (Swenberg et al. 1988). Accumulation of the chemical $_{\text{alpha }2}\mu$-globulin complex causes lysosomal protein overload and necrosis of the cells, with subsequent cellular regeneration that continues as long as the rat is exposed to the chemical and produces $_{\text{alpha }2}\mu$-globulin. The increased cellular proliferation may promote tumorigenesis by increasing the number of cells in the kidney that have undergone spontaneous initiation. Given the findings of Strasser (1988) that isophorone was associated with $_{\text{alpha }2}\mu$-globulin and induced protein droplet formation in the kidneys of male rats and that cell proliferation may be involved in the mechanism of male rat kidney tumorigenesis, the finding in the NTP (1986)

study of increased incidences of tubular epithelial hyperplasia in addition to the increased incidences of tubular cell tumors is consistent with the mechanism proposed by Swenberg et al. (1989).

As discussed above, $_{alpha\ 2}\mu$-globulin appears to be unique to male rats. If isophorone induces kidney tumors solely by the suggested mechanism, the absence of $_{alpha\ 2}\mu$-globulin in humans raises the question of the relevance to humans of the isophorone-induced kidney tumors in male rats. Although $_{alpha\ 2}\mu$ globulin is related to other low molecular weight transport proteins that have been detected in humans (Swenberg et al. 1989), it is not known whether chemicals that produce kidney tumors in male rats bind to the human proteins or produce similar effects in humans. This issue is currently being reviewed by the EPA.

Male rats treated with isophorone in the NTP (1986) study also had preputial gland carcinomas, an extremely rare finding. Analogous tissues in humans are modified sebaceous glands in the prepuce (foreskin of the penis), but it is not known whether isophorone could cause tumors in these glands or other glandular tissues in humans.

In male mice, significant dose-related trends by the Cochran-Armitage test were found for hepatocellular adenoma and carcinoma (combined) and for fibromas, sarcomas, fibrosarcomas, and neurofibrosarcomas (combined) of the integumentary system. These incidences were increased significantly above control rates in the high-dose males by the Incidental Tumor and Fisher Exact tests. Low-dose male mice had significantly increased incidences of lymphoma compared with controls by the Life Table test and Fisher Exact test, but the tests for dose-related trend were not significant because the incidence in high-dose male mice was lower than the incidence in low-dose male mice. This evidence was considered equivocal by NTP (1986), and it is not known whether exposure to isophorone would cause cancer in human.

2.4 LEVELS IN HUMAN TISSUES AND FLUIDS ASSOCIATED WITH HFALTH EFFECTS

No studies were located regarding levels of isophorone or its metabolites in human tissues and fluids associated with effects. Furthermore, no studies were located describing methods for detecting isophorone or its metabolites in human tissues and fluids.

2.5 LEVELS IN THE ENVIRONMENT ASSOCIATED WITH LEVELS IN HUMAN TISSUES AND/OR HEALTH EFFECTS

Although isophorone has been detected in environmental media (see Chapter 5), no data were located allowing associations of effects of isophorone in humans or levels of isophorone or its metabolites in human tissues and fluids with environmental levels of isophorone.

2. HEALTH EFFECTS

2.6 TOXICOKINETICS

2.6.1 Absorption

2.6.1.1 Inhalation Exposure

No studies were located regarding the rate and extent of absorption of isophorone following inhalation exposure of humans or animals to isophorone. Isophorone was widely distributed to the organs of rats exposed for 4 hours to a concentration of 400 ppm isophorone (Dutertre-Catella 1976), indicating that isophorone is absorbed after inhalation exposure. That isophorone is absorbed by the lungs can also be inferred from the systemic toxicity observed in animals following inhalation exposure (see Section 2.2.1.2 on systemic effects following inhalation exposure). Imbriani et al. (19'85) measured a blood/air partition coefficient of 2349 for isophorone, indicating that isophorone is absorbed readily from the lungs.

2.6.1.2 Oral Exposure

No studies were located regarding the absorption of isophorone in humans following oral exposure.

Preliminary results of a pharmacokinetic study indicate that rats treated orally with ^{14}C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (Strasser 1988). The majority was found in the urine indicating that isophorone was well absorbed. The wide distribution of isophorone in the organs of rats and a rabbit l-5 hours after dosing by gavage with 4000 mg/kg (Dutertre-Catella 1976) indicates rapid gastrointestinal absorption. In two rabbits given a gavage dose of 1000 mg/kg isophorone, a blood level of isophorone of 102 μg/L was found within 10 minutes. The level increased to 141 μg/L in 30 minutes and declined to ≤0.05 μg/L in 21 hours. The results indicate rapid absorption and elimination. The detection of unchanged isophorone and its metabolites (see Section 2.6.3 on Metabolism) in the urine and the observations of . systemic toxicity and carcinogenicity (see Section 2.2.2 on effects of oral exposure) in animals exposed orally to isophorone provide qualitative evidence that isophorone is absorbed after oral exposure.

2.6.1.3 Dermal Exposure

No studies were located regarding the absorption of isophorone following dermal exposure of humans or animals. A report that a high dermal dose resulted in signs of CNS depression in l/4 rabbits suggests that isophorone is absorbed dermally (Hazleton Labs 1964), but other systemic effects have not been described.

2.6.2 Distribution

2.6.2.1 Inhalation Exposure

No studies were located regarding the distribution of isophorone following inhalation exposure of humans.

In rats exposed to 400 ppm isophorone for 4 hours and sacrificed immediately after exposure or 1.5 or 3 hours after exposure, levels of isophorone were highest in all tissues examined (brain, lungs, heart, stomach, liver, spleen, pancreas, kidney, adrenals, testicles, and ovaries) immediately after exposure (Dutertre-Catella 1976). Levels ranged from 1.5 to 74 μg/g tissue wet weight. The levels dec1ine.d rapidly in males but declined very little in females by 3 hours after exposure.

2.6.2.2 Oral Exposure

No studies were located regarding the distribution of isophorone in humans following oral exposure.
Radiolabel was widely distributed in male rats 24 hours after an oral dose of ^{14}C-isophorone in corn oil, with highest levels in the liver, kidney, preputial gland, testes, brain, and lungs (Strasser 1988). Isophorone was widely distributed to the tissues of rats and a rabbit following treatment with isophorone at a gavage dose of 4000 mg/kg (Dutertre-Catella 1976). The rats died within 1-5 hours and the rabbit died within an hour after dosing at which times the tissues were sampled for analysis. In rats, tissue levels of isophorone in μg/g tissue wet weight were as follows: stomach - 6213, pancreas - 2388, adrenals - 1513, spleen - 1038, liver - 613, brain - 378, lung - 383, heart - 387, kidney - 465, testes - 275, and ovaries - 471. In the rabbit, tissue levels were as follows: stomach - 5395, adrenals - 1145, ovaries - 3000, spleen - 545, liver - 515, kidney - 295, heart - 260, and lungs - 50.

2.6.2.3 Dermal Exposure

No studies were located regarding the distribution of isophorone following dermal exposure of humans or animals.

2.6.3 Metabolism

No studies were located regarding the metabolism of isophorone in humans following exposure to isophorone by any route.

Rabbits and rats treated orally with isophorone excreted unchanged isophorone in the expired air and in the urine (Dutertre-Catella et al. 1978; Truhaut et al. 1970). The urine also contained 3-carboxy-5,5-dimethyl-2-cyclohexene-l-one and glucuronic conjugates of 3,3,5-trimethyl-2-cyclohexene-l-01 (isophorol), 3,5,5-trimethylcyclohexanone

(dihydroisophorone), and cis- and trans-3,5,5 trimethylcyclohexanols. Rat urine contained more dihydroisophorone and less isophorol than did rabbit urine. Dutertre-Catella et al. (1978) proposed that metabolism of isophorone involves methyloxidation to 3-carboxy-5,5-dimethyl-2-cyclohexene-l-one, reduction of the ketone group to isophorol, reduction'of the ring double bond to dihydroisophorone, and dismutation of dihydroisophorone to cis- and trans-3,5,5-trimethylcyclohexanols. The metabolic pathways are presented in Figure 2-3.

2.6.4 Excretion

2.6.4.1 Inhalation Exposure

No studies were located regarding the excretion of isophorone or its metabolites following inhalation exposure of humans to isophorone. Dutertre-Catella (1976) found that the excretion of isophorone in air was low (110 μg) and declined further to 30 μg at 2.5-3 hours after exposure of rats to 400 ppm for 4 hours.

2.6.4.2 Oral Exposure

No studies were located regarding the excretion of isophorone or its metabolites following oral exposure of human to isophorone.
Rats and rabbits excreted unchanged isophorone and metabolites in the urine and unchanged isophorone in the expired air following oral dosing with isophorone (Dutertre-Catella et al. 1978), but the rate and extent of excretion were not reported. Preliminar results of a pharmacokinetic study indicate that following an oral dose of ^{14}C-isophorone, male rats excreted 93% of the radiolabel in the urine, feces, and expired air in 24 hours, with the majority in the urine (Strasser 1988).

2.6.4.3 Dermal Exposure

No studies were located regarding the excretion of isophorone or its metabolites following dermal exposure of humans or animals.

2.7 INTERACTIONS WITH OTHER CHEMICALS

The possible synergistic interactions of isophorone with other solvents are important because mixed exposures occur in occupational settings and may occur in the environment. The joint toxicity of isophorone with 26 other industrial liquid chemicals based on determinations of the oral LD_{5O}s in rats of each chemical alone and in a 1:l (v/v) mixture was determined (Smyth et al. 1969). The LD5Os of the mixtures were predicted based on the assumption of additivity of the LD_{5O}s of each component, and the ratios of the predicted values to experimentally determined values were calculated. Greater than additive toxicity was observed for the mixtures of isophorone with nine chemicals: tetrachloroethylene, propylene glycol,

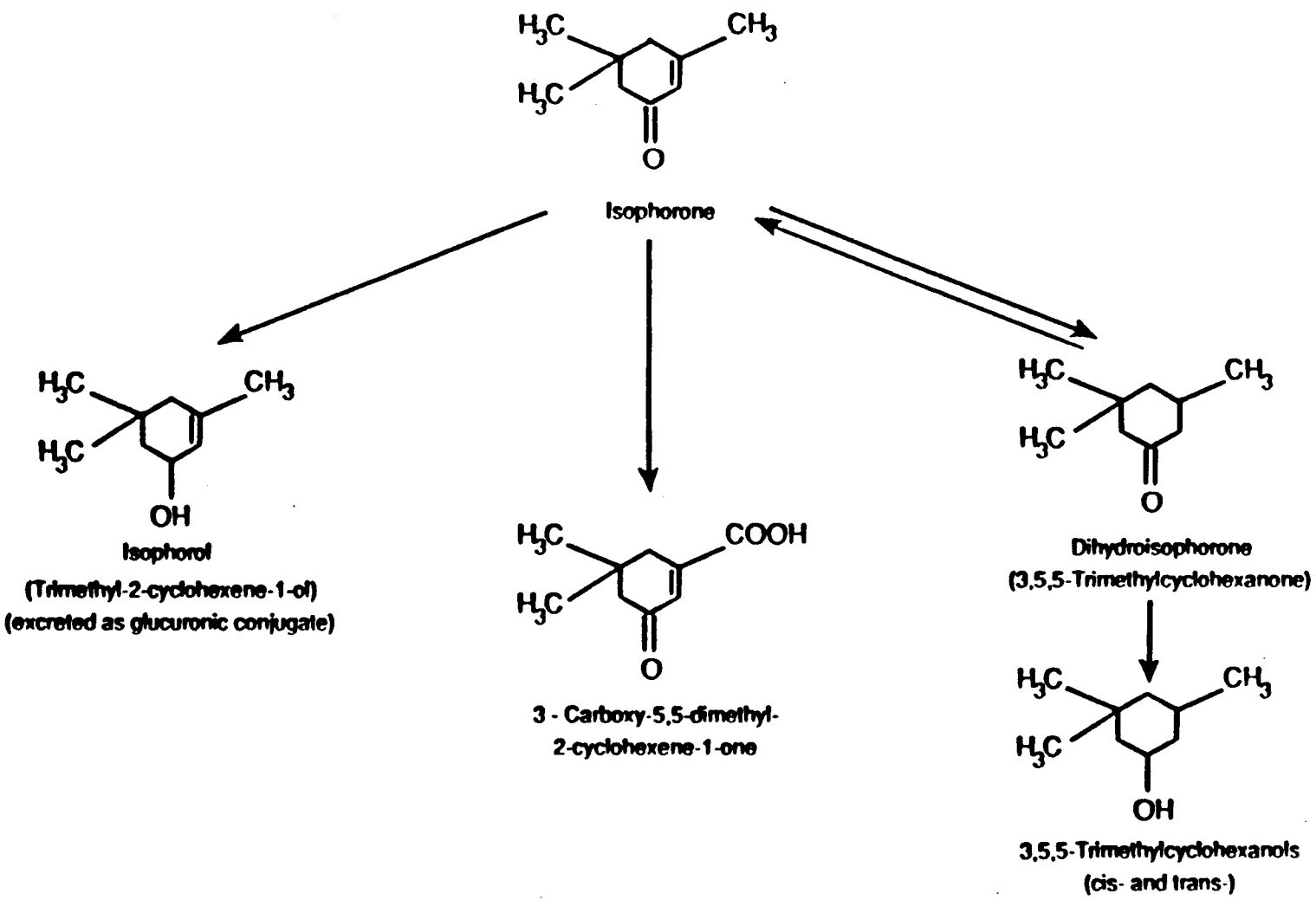

FIGURE 2-3. Metabolic Scheme for Isophorone

Source: Dutertre-Catella et al. 1978.

morpholine, ethyl alcohol, ethyl acetate, carbon tetrachloride, acrylonitrile, acetonitrile, and acetone. Less than additive toxicity was observed for the mixtures of isophorone with 17 chemicals: Ucon LB-250, Ucon 50-HB-260, toluene, Tergitol XD, propylene oxide, polyethylene glycol 200, Phenyl Cellosolve, nitrobenzene, acetophenone, aniline, Butyl Cellosolve, butyl ether, diethanolamine, dioxane, ethyl acrylate, ethylene glycol I and formalin. When the frequency distribution of the ratios for all combinations of all chemicals were adjusted to give a normal distribution, however, none of the ratios for mixtures with isophorone deviated significantly from the mean ratios, indicating essentially additive toxicity. In a subsequent study, the additivity of equitoxic mixtures, defined as a mixture of chemicals in volumes directly proportional to their oral LD50 in rats, was determined (Smyth et al. 1970). Isophorone showed less than additive toxicity with Phenyl Cellosolve and Ucon Fluid 50-HB-260, and greater than additive toxicity with propylene oxide. The mechanism for such interactions is not known.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Isophorone produced kidney effects in male rats in the NTP (1986) study. Strasser (1988) found that isophorone caused protein droplet formation in the kidneys of male rats, suggesting that isophorone can induce protein nephropathy. Alden (1986) discussed the possibility that proteinuric humans and humans with low molecular weight protein nephropathy, such as people with multiple myeloma (Bence-Jones protein) or mononuclear cell leukemia (lysozyme), may be more susceptible to chemically-induced protein nephropathy. He concluded, however, that this syndrome is probably specific to the male rat.

2.9 ADEQUACY OF THE DATABASE

Section 104 (i) (5) of CERCLA, 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 isophorone is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been Profiled.

2.9.1 Existing Information on Health Effects of Isophorone

As seen from Figure 2-4, very little information is available regarding the health effects of exposure of humans to isophorone.

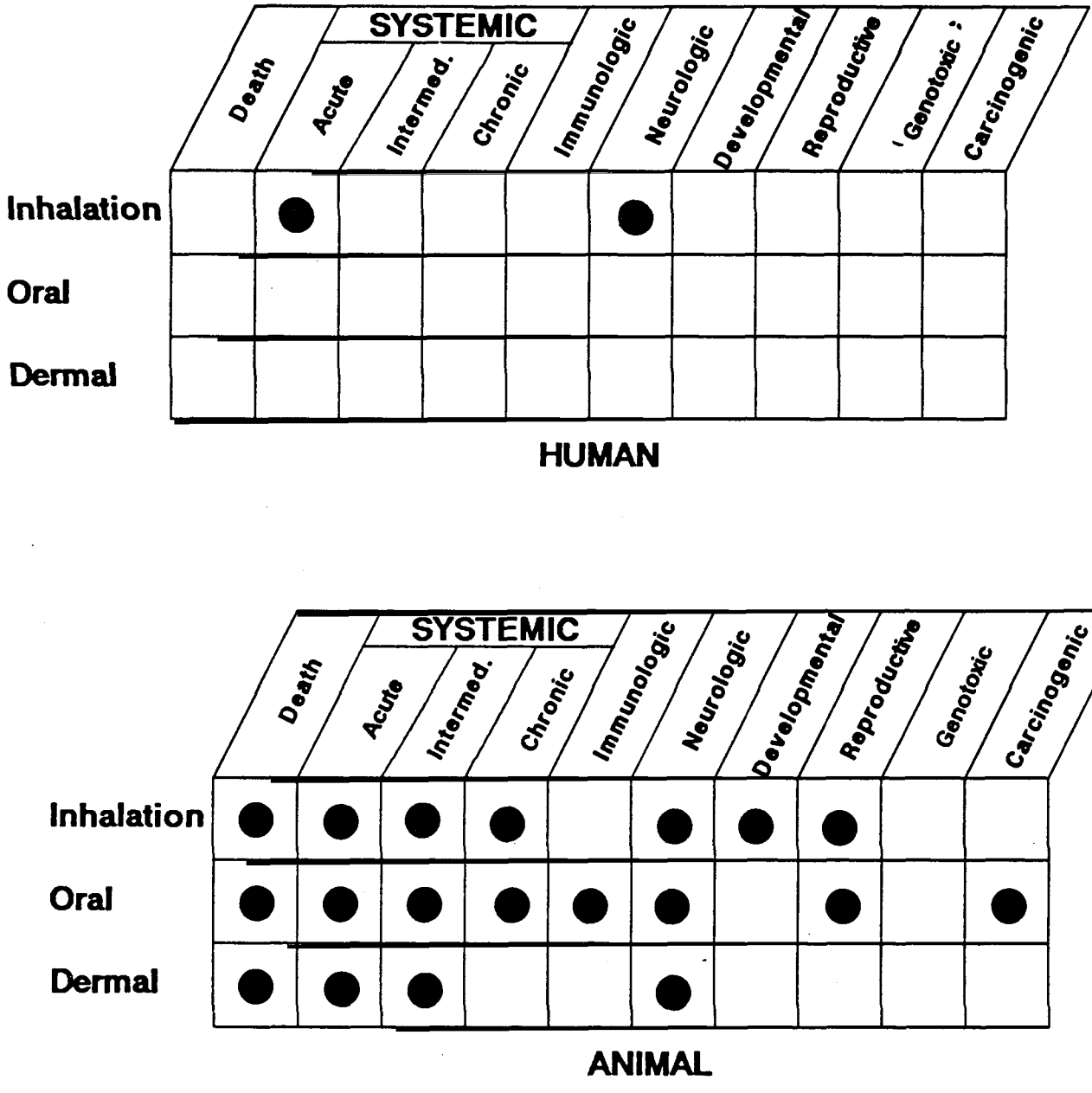

FIGURE 2-4 Existing Information on Health Effects of Isophorone

Experimental studies in humans have attempted to determine the inhalation thresholds for odor detection and eye, nose, and throat irritation. Reports on humans occupationally exposed to isophorone also indicate that isophorone is irritating to the skin, eye, nose, and throat, and may cause symptoms of dizziness, fatigue, and malaise.

Data are available for acute and intermediate inhalation exposures that have resulted in death of animals. These exposures also produced signs of central nervous system toxicity, lung irritation, possible kidney damage, and possible hematological changes and growth depression. Chronic inhalation exposure of rats and rabbits resulted in mild liver effects. Inhalation exposure of pregnant rats and mice during gestation did not result in fetotoxic or teratogenic effects at concentrations up to 115 ppm, but results at 150 ppm are difficult to interpret. No information was available on the effects of chronic inhalation exposure.

Data are available for oral doses associated with death and increased mortality in acute, intermediate, and chronic exposure. Acute and intermediate oral studies have also produced signs of central nervous system toxicity at high doses during exposure. In intermediate duration studies of oral exposure of animals to isophorone, comprehensive histological examination of tissues and organs found no effects. Chronic oral exposure of mice resulted in hyperkeratosis of the forestomach, non-neoplastic liver lesions, and equivocal evidence of liver tumors, integumentary system tumors, and malignant lymphomas. Chronic oral exposure of rats resulted in hyperplastic and neoplastic kidney lesions and preputial gland carcinomas.

Application of isophorone to the skin of animals results in skin irritation, and application to the eye results in severe eye damage. There is some information that dermal exposure of rabbits causes signs of neurotoxicity at high doses. This could indicate that isophorone is absorbed dermally, but other systemic effects have not been described.

No studies of the genotoxic effects of oral, inhalation, or dermal exposure to isophorone were found, but studies in bacteria and mammalian cells indicate that isophorone is at best weakly genotoxic.

2.9.2 Data Needs

Single Dose Exposure. Studies of single inhalation, oral, or dermal exposure of rats, guinea pigs, and mice have provided data on lethal and non-lethal levels of isophorone and levels producing signs of neurotoxicity. Single-dose dermal and ocular studies in animals have demonstrated that isophorone is irritating to the skin and eyes. Gross clinical and necropsy observations have been made, but no reliable single-dose study examined the internal tissues of animals histologically or attempted to identify doseresponse data for more subtle systemic toxic effects, Such studies might provide information on the mechanisms of lethality and neurotoxicity, as

well as information on the thresholds for systemic toxicity due to single dose exposure.

Repeated Dose Exposure. Repeated inhalation exposure studies of isophorone conducted in rats and guinea pigs by Smyth et al. (1942) reported severe respiratory and kidney lesions, but this study was criticized by Rowe and Wolf (1963) for using impure isophorone and overestimating the concentrations. Furthermore, dose-response data were poorly reported and results in rats and guinea pigs were reported together. A study by Hazleton Labs (1968) suggested hematological and liver weight effects in rats, but only one exposure concentration was used. A 4-6 month inhalation study in rats reported ocular and nasal irritation but no exposure-related effects on lungs or livers (Dutertre-Catella 1976). Results were poorly reported and only one concentration was used. A wellconducted subchronic inhalation study that uses several concentrations of pure isophorone and monitors for clinical signs, hematological and biochemical changes, and gross and histopathological changes in animals would provide dose-response data for toxicological endpoints and remove uncertainties associated with the study by Smyth et al. (1942). Wellconducted, repeated-dose oral studies in rats, mice, and dogs at several dosage levels including maximum tolerated doses produced no systemic effects. A 2-week dermal study in rabbits revealed no biochemical evidence of liver damage (Dutertre-Catella 1976), but other indices of toxicity were not examined. An a-week dermal study in rats revealed erythema and scar tissue formation, which disappeared after exposure ceased (Dutertre-Catella 1976). As screen printers are repeatedly exposed dermally to isophorone and the extent of dermal absorption is not known, better repeated dermal dose studies examining systemic toxicity in animals might provide information on whether repeated dermal exposure of humans poses a threat of toxic potential.

Chronic Exposure and Carcinogenicity. Well-conducted chronic oral studies provide information on the systemic and carcinogenic effects of isophorone in rodents. In a chronic oral study, male rats exposed to isophorone developed kidney and preputial gland tumors. The relevance of these tumors in male rats to humans has been questioned; therefore, additional research to clarify the relevance is desirable. Indeed, on-going studies are being conducted (see Section 2.9.3). In a chronic inhalation study, rats and rabbits had ocular and nasal irritation and slight liver effects (Dutertre-Catella 1976), but few animals and only one concentration were used. No chronic dermal studies were located. It is not possible to predict that effects following chronic inhalation or dermal exposure to isophorone would be similar to those following chronic oral exposure, partially because the pharmacokinetic disposition of isophorone has not been compared for the three routes of exposure. Available toxicokinetic data (see Section 2.6) indicate that isophorone is metabolized to dihydroisophorone, isophorol, and other products after oral dosing of rats and rabbits, but different metabolic pathways may operate following inhalation and dermal exposure, Differences in absorption and tissue

distribution among the three routes of exposure could also account for differences in toxic response. Chronic inhalation and dermal studies in animals might provide dose-response data on the systemic effects that could be related to possible systemic effects of inhalation and dermal exposure of humans. Long-term exposure of humans to isophorone by inhalation and by skin contact occurs in occupational settings.

Genotoxicity. The available genotoxicity studies (Salmonella/microsome assays, mutations in mouse lymphoma cells, tests of unscheduled DNA synthesis, sister chromatid exchange, and chromosome aberrations in cultured mammalian cells, and an in vivo micronucleus tests) indicate that isophorone may be weakly genotoxic. Additional genotoxicity tests would add to the rather limited data base on genotoxicity, but probably would not change the conclusion that isophorone in weakly genotoxic.

Reproductive Toxicity. An intermediate duration study examined only the pregnancy rate and litter size in rats exposed to isophorone by inhalation for 3 months before mating. Histological examination of reproductive organs of rats, mice, and dogs exposed orally to isophorone in subchronic and chronic studies indicate no treatment-related lesions, but multigeneration or continuous breeding studies have not been conducted. Such studies would provide further information regarding the reproductive effects of isophorone in animals, which may then be related to possible reproductive effects in humans.

Developmental Toxicity. Developmental studies by the inhalation route in rats indicated growth retardation in the rat fetuses at a concentration of 115 ppm, and maternal toxicity at all concentrations tested (≥25 ppm). Exencephaly was seen in several rat and mouse fetuses after exposure of the dams to 150 ppm during the organogenesis period. The developmental effects following oral or dermal exposure have not been studied. It is not known whether isophorone crosses the placenta, but there is no reason to assume that it would not do so. Further developmental studies in animals by relevant environmental routes, such as drinking water and diet, would provide information on possible fetotoxic and teratogenic effects in animals that might be relevant to humans. Studies in drinking water and diet are particularly relevant because isophorone has been detected in groundwater, ambient water, drinking water, oysters, and cranberries (see Section 5.4 on environmental monitoring).

Immunotoxicity. No histopathological effects on immunological organs and tissues of animals were found in subchronic and chronic oral studies, but a battery of immunotoxicity tests has not been performed. Such tests provide a more sensitive assessment of possible immunotoxic effects than do histological examinations of tissues and organs of the immunological system. Isophorone is a skin irritant in rabbits, guinea pigs, and humans, but it has not been tested for sensitization. Such tests might provide information on whether an allergic response to isophorone is likely. The potential for

dermal contact by humans occurs in occupational settings and in soil at waste sites.

Neurotoxicity. No histopathological effects on organs and tissues of the neurological systems of animals were found in subchronic and chronic oral studies, but signs of central nervous system toxicity were reported in inhalation, oral, and dermal studies. A battery of tests for neurotoxicity would provide further information of the neurotoxicity in animals, which then might be related to possible neurotoxic effects in humans. Epidemiological and Human Dosimetry Studies. The only known health effects of isophorone in humans are eye, nose, and throat irritation, and fatigue and malaise. This information comes from two limited industrial hygiene surveys, two experimental studies in human volunteers, and a communication to the ACGIH. Effects in animals, however, include CNS depression, liver and kidney damage, hyperkeratosis of the forestomach, some evidence of cancer, and suggestive evidence of developmental toxicity. As discussed in Chapter 5, isophorone has been detected in surface water, drinking water, industrial effluents, urban runoff, and water and soil at waste sites. Isophorone has a relatively low vapor pressure (Union Carbide 1968) and high reactivity with hydroxyl radicals (Atkinson 1985, 1987); therefore, exposure to isophorone in the ambient atmosphere distant from the source is unlikely. Indeed, monitoring data for isophorone in air are lacking. Inhalation as well as dermal exposure, however, occurs in occupational settings where isophorone is used as a solvent. Epidemiology studies of people who live in areas where isophorone has been detected in ambient and drinking water, near industries releasing isophorone, or near hazardous waste sites, and of people occupationally exposed, could provide information on whether isophorone produces effects in humans similar to those seen in animals, or other toxic effects.

No studies were located that monitored human tissues for isophorone or its metabolites. Furthermore, analytical methods for the detection of isophorone or its metabolites in humans tissues and fluids were not located. Metabolism studies in rats and rabbits, however, indicated that isophorone, isophorol, dihydroisophorone, 3-carboxy-5,5-dimethyl-2-cyclohexene-l-one, and cis- and trans-3,5,5-trimethylcyclohexanols were excreted in the urine following oral exposure to isophorone (Dutertre-Catella et al. 1978). If isophorone and these or other metabolites can be detected in the urine of humans and be correlated with exposure, it may be possible to monitor humans for exposure. If toxic effects of isophorone are identified in humans, it may then be possible to correlate urinary levels of isophorone or its metabolites with systemic effects.

Biomarkers of Disease. No disease states in humans produced by exposure to isophorone are known. If epidemiological studies are conducted that correlate exposure with diseases, it may be possible to identify subtle changes associated with a particular disease state.

2. HEALTH EFFECTS

Disease Registries. At present, the only known health effects of isophorone in humans are eye, nose, and throat irritation, and fatigue and malaise. If epidemiological studies identify particular diseases produced by isophorone, it may be possible to determine the number of people affected and the factors associated with identifying the disease in certain populations, such as exposure to high levels near hazardous waste sites.

Bioavailability from Environmental Media. No studies were located regarding the bioavailability of isophorone from environmental media. Furthermore, no reports were located indicating that isophorone or its metabolites have been detected in human tissues or fluids. Since the monitoring literature reports that isophorone is present in the environment as well as in environmental organisms, the lack of data does not necessarily indicate a lack of bioavailability. Fish may be the only source of isophorone in the environment that is not subject to large spatial and temporal variations in concentration, as appears to be the case with drinking water. In particular, fish in the Lake Michigan area are known to contain isophorone (Camanzo et al. 1987), and analysis of the body fluids of people who consume the fish may allow a determination of the existence of exposure and an estimation of the degree of exposure.

Food Chain Bioaccumulation. No studies were located regarding the food chain bioaccumulation of isophorone from environmental media. The monitoring literature reports that isophorone is present in the environment as well as in environmental organisms. The monitoring data further suggest that isophorone levels in fish do not correlate well with the lipid content of the fish (see Section 5.4). Thus, structure-activity relationships developed to estimate levels in biological media based on the partitioning properties of a chemical may not provide accurate information for isophorone. Furthermore, only one bioaccumulation study was available. In this study, which indicated a low potential for bioaccumulation, fish were exposed to isophorone in water rather than in food. From these data, it appears that food chain bioaccumulation may be occurring, and a clearer understanding of the potential for this would aid in determining how levels in the environment affect the food chain and potentially impact on human exposure levels.

Absorption, Distribution, Metabolism, Excretion. The only toxicokinetic data of isophorone are the in vivo metabolism studies in rats and rabbits following oral exposure (Dutertre-Catella et al. 1978) and the preliminary disposition data of Strasser (1988). These studies indicate that isophorone is metabolized to dihydroisophorone and isophorol in animals following oral exposure. Different metabolic pathways and patterns of distribution and excretion, however, may operate after inhalation or dermal exposure. Differences in the rate and extent of absorption, metabolic pathways, and disposition may account for differences in the toxicity of a chemical following exposure by different routes. Thus, further studies in animals of the rate and extent of absorption and excretion following exposure by all three routes and of distribution and metabolism following

inhalation and dermal exposure, and in vitro studies to elucidate metabolic pathways would provide the information to fully characterize the pharmacokinetics of isophorone in animals. Ethical considerations limit the testing of humans, but the determination of the urinary excretion of isophorone and its metabolites by humans with known exposure to isophorone (e.g., workers in the printing trades), may provide a means of monitoring humans for exposure.

Comparative Toxicokinetics. The metabolism studies by Truhaut et al. (1970) and Dutertre-Catella et al. (1978) indicated that metabolism of isophorone in rats and rabbits was qualitatively similar, but the proportion of the metabolites excreted was different. Differences in the toxicokinetics of a chemical among species may account for differences in toxic responses. The potential for isophorone to produce toxic effects has been investigated in rats, mice, dogs, guinea pigs, and rabbits, but the animal species that serves as the best model for extrapolating results to humans is not known. Ethical considerations limit the amount of information that can be obtained by testing isophorone in humans, but analysis of the urine of people with known exposure to isophorone for parent compound or metabolites could provide knowledge of the metabolic pathways in humans. Qualitative and quantitative comparison of human metabolites with those of animals could help identify the most appropriate species to serve as a model for predicting toxic effects in humans and studying the mechanisms of action.

2.9.3 On-going Studies

A manuscript of the study demonstrating protein droplet formation in the kidneys of male rats following acute oral exposure to isophorone and of the distribution study by Strasser will be submitted for publication to Toxicology and Applied Pharmacology (Strasser 1988). These studies were presented as a Poster Presentation at the Society of Toxicology meetings in February, 1988, and an abstract (Strasser et al. 1988) has been published. In addition, the manuscript to be submitted to Toxicology and Applied Pharmacology will contain added information on the distribution of radiolabel in female rats and in the preputial gland of male rats (Strasser 1988).

James Swenberg, formerly at the Chemical Industry Institute of Toxicology (CIIT), and his colleagues at CIIT are continuing the investigation of the mechanism of hydrocarbon-induced nephropathy and the induction of renal tumors in male rats (Swenberg et al. 1989). These investigations include isophorone. Swenberg and colleagues are also investigating whether low molecular weight proteins found in humans behave similarly to alpha 2µ-globulin of male F344 rats.

No on-going biomonitoring studies or studies of toxic effects of isophorone in humans were identified.