2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of endosulfan. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

Technical-grade endosulfan contains at least 94% α-endosulfan and β-endosulfan. The α- and β-isomers are present in the ratio of 7:3, respectively. The majority of the studies discussed below used technical-grade endosulfan. However, a few examined the effects of the pure α- and β-isomers. Endosulfan sulfate is a reaction product found in technical-grade endosulfan as a result of oxidation, biotransformation, or photolysis. There is very little difference in toxicity between endosulfan and its metabolite, endosulfan sulfate. However, the α-isomer has been shown to be about three times as toxic as the β-isomer of endosulfan.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a

considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for endosulfan. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

Limited information is available regarding the effects of endosulfan in humans and animals after inhalation exposure. The reports of effects in humans are limited to case reports of adverse effects noted in workers exposed to large quantities of endosulfan during its manufacture. Exposures in these reports are likely to be a combination of inhalation and dermal exposures. Therefore, the findings from these case reports are also presented in the section on dermal exposures (Section 2.2.3).

2.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to endosulfan. LC_{50} (lethal concentration, 50% kill) values of 12.6 mg/m^3 and 34.5 mg/m^3 for female and male rats, respectively, were obtained after a 4-hour nose-only exposure to aerosolized endosulfan (Hoechst 1983a). No deaths were observed among male and female rats exposed to aerosolized endosulfan (nose-only) at concentrations as high as 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c). Acute LC_{50} values for male and female rats are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

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

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to endosulfan.

Irregular respiration was observed in both male and female rats after a 4-hour nose-only inhalation exposure to aerosolized endosulfan (Hoechst 1983a). In both male and female rats, dyspnea was observed at the lowest concentrations tested (12.3 and 3.6 mg/m^3 for males and females, respectively). Autopsies of the rats that died revealed dark-red, pinhead-sized foci on the lungs. It is unclear whether these effects represent direct effects of inhaled endosulfan on respiratory tissues or whether they are secondary to central nervous system effects on respiratory function. No treatment-related effects were

Table 2-1. Levels of Significant Exposure to Endosulfan - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference Chemical Form
					Less serious (mg/m3)	Serious (mg/m3)	
ACUTE EXPOSURE							
Death							
1	Rat (Wistar)	4 hr				12.6 F (LC_{50})	Hoechst 1983a Technical
2	Rat (Wistar)	4 hr				34.5 M (LC_{50})	Hoechst 1983a Technical
Systemic							
3	Rat (Wistar)	4 hr	Resp			3.6 F (dyspnea)	Hoechst 1983a Technical
Neurological							
4	Rat (Wistar)	4 hr				3.6 F (trembling; ataxia)	Hoechst 1983a Technical

Table 2-1. Levels of Significant Exposure to Endosulfan - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
5	Rat (Wistar)	21 d 5 d/wk 6 hr/d	Resp	2.0			Hoechst 1984c Technical
			Cardio	2.0			
			Gastro	2.0			
			Hemato	2.0			
			Musc/skel	2.0			
			Hepatic	2.0			
			Renal	2.0			
			Endocr	2.0			
			Dermal	2.0			
			Ocular	2.0			
			Bd Wt		2.0 M (decreased body weight gain)		
				2.0 F			
Immunological/Lymphoreticular							
6	Rat (Wistar)	29 d 5 d/wk 6 hr/d		2.0			Hoechst 1984c Technical
Neurological							
7	Rat (Wistar)	21 d 5 d/wk 6 hr/d		2.0			Hoechst 1984c Technical

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest- observable-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; wk = week(s).

Figure 2-1. Levels of Significant Exposure to Endosulfan - Inhalation

Acute (≤14 days)

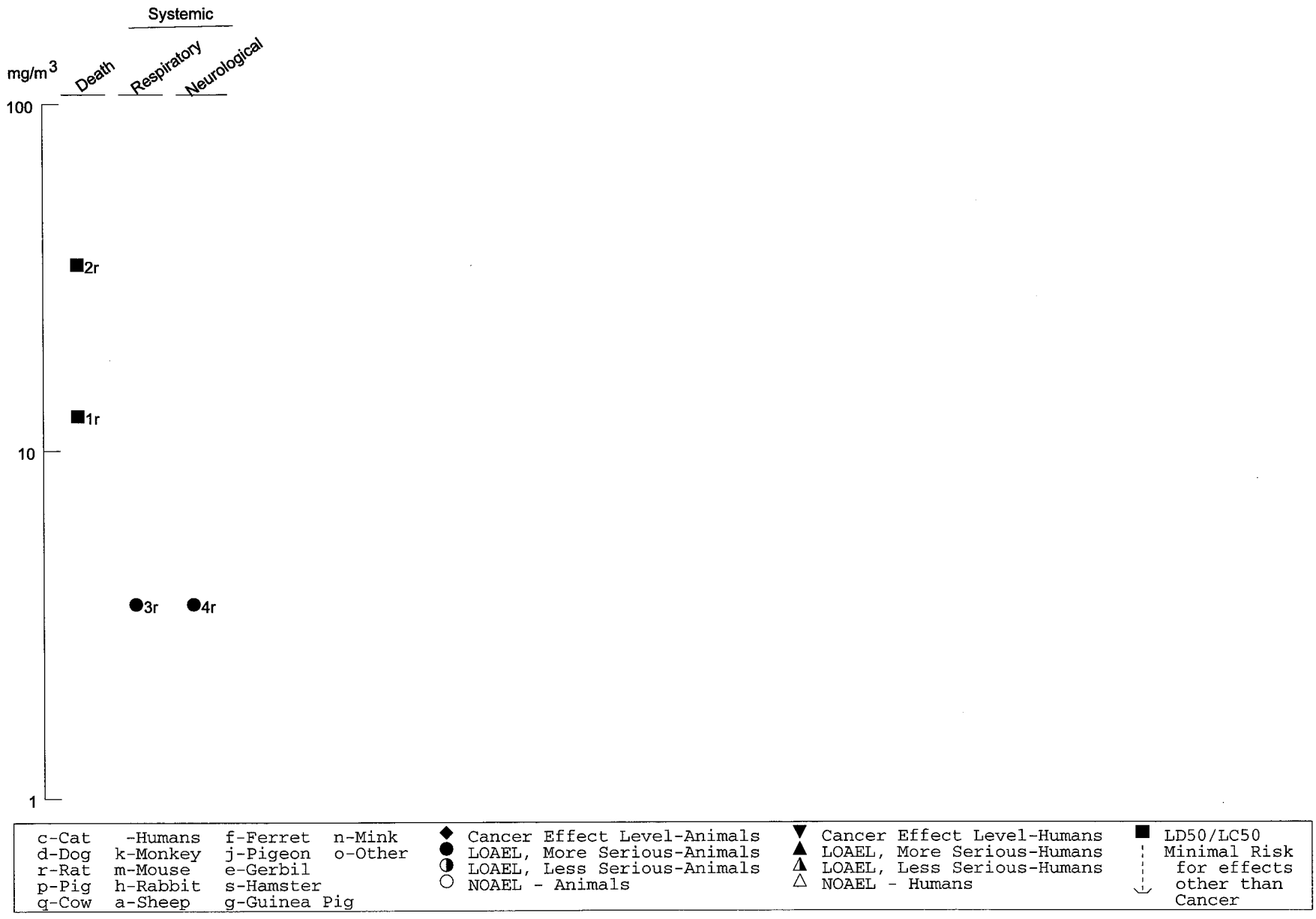
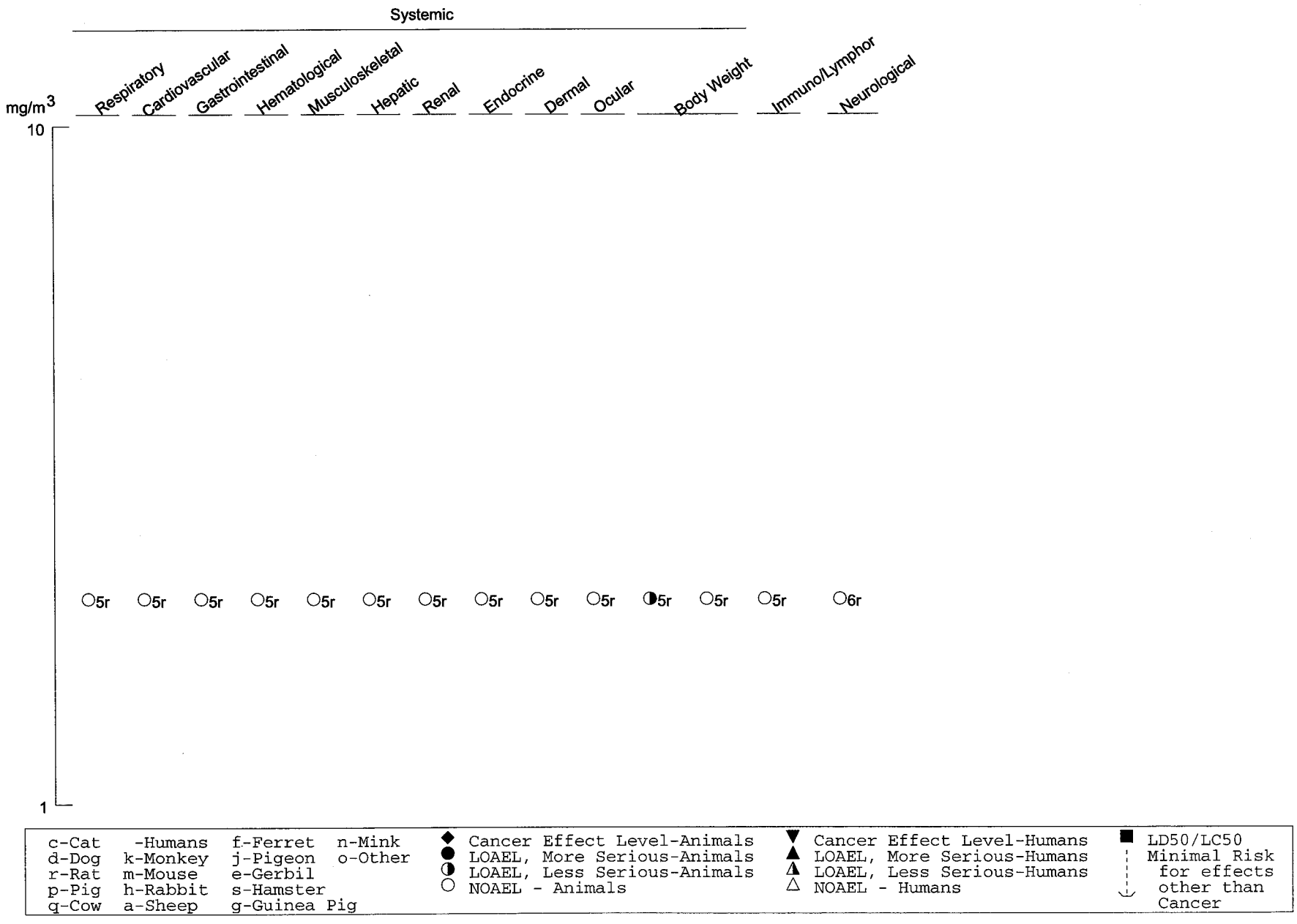


Figure 2-1. Levels of Significant Exposure to Endosulfan - Inhalation (Continued)

Intermediate (15-364 days)



revealed by routine gross and histopathologic examination of the nasal cavity, trachea, and lungs of male and female rats exposed (nose-only) to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to endosulfan. Routine gross and histopathologic examination of the heart and aorta of rats exposed (nose-only) to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days revealed no treatment-related effects (Hoechst 1984c).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to endosulfan. Routine gross and histopathologic examination of tissues of the gastrointestinal system (parotid and submandibular glands, esophagus, stomach, small and large intestines, and pancreas) revealed no treatment-related effects following exposure to aerosolized endosulfan for 6 hours/day, 5 days/week for a total of 21 out of 29 days at concentrations of up to 2 mg/m^3 (Hoechst 1984c).

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of hematopoietic organs (spleen and bone marrow) and routine hematological analyses did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of skeletal muscle and the diaphragm revealed no treatment-related effects following nose-only inhalation exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of the liver did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of the kidneys and urinary bladder did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Endocrine Effects. No studies were located regarding endocrine effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of the pituitary gland did not reveal any effects in rats exposed nose-only to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to endosulfan. Routine gross and histopathologic examination of the skin did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Ocular Effects. No studies were located regarding ocular effects in humans after inhalation exposure to endosulfan. Routine gross and histopathologic examination of the eyes did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c).

Body Weight Effects. No studies were located regarding body weight effects in humans after inhalation exposure to endosulfan. Body weight gain was significantly reduced in male, but not female, rats exposed nose-only to concentrations of endosulfan of 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c). No significant effect was seen at an exposure level of 1 mg/m^3.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after inhalation exposure to endosulfan.

Routine gross and histopathologic examination of the lymph nodes, thymus, and spleen did not reveal any effects of nose-only exposure of rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c). No studies directly assessing immunologic function were located.

2.2.1.4 Neurological Effects

Neurotoxicity is the primary effect observed in humans following occupational exposure to endosulfan. Convulsions were reported in nine individuals exposed to the endosulfan-containing insecticide Thiodan® during bagging (Ely et al. 1967). Other effects noted in at least one of the subjects prior to the onset of convulsions included malaise, nausea, vomiting, dizziness, confusion, and/or weakness. In addition, a case of long-term, possibly permanent brain damage in an industrial worker was attributed by Aleksandrowicz (1979) to endosulfan exposure. This worker was exposed by cleaning vats that contained residues of endosulfan solution. The acute phase of the poisoning was manifested by repeated convulsions and impaired consciousness. After recovery from the repeated seizure episode, the patient became disoriented and agitated. Two years later, he exhibited cognitive and emotional deterioration, memory impairment, and impairment of visual-motor coordination manifested by an inability to perform small tasks. However, modest alcohol consumption (1L of wine consumed per week) may have been a contributing factor. Limitations associated with these reports include lack of quantitative exposure data, lack of data on the duration of exposure, the possibility of multiple routes of exposure (i.e., oral and dermal, as well as inhalation) and possible concurrent exposure to other chemicals. Therefore, this information can only provide qualitative evidence of neurotoxicity associated with inhalation exposure to endosulfan in humans.

Evidence of neurotoxicity was also observed in animal studies. Nose-only exposure of rats to endosulfan at concentrations of 3.6 mg/m^3 in females and 12.3 mg/m^3 in males resulted in trembling and ataxia (Hoechst 1983a). At higher concentrations in both sexes, tremors, tonic-clonic convulsions, and reduced corneal, pupillary, placing, shock, paw-pinch, and cutaneous reflexes were observed. Nose-only exposure of male and female rats to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for

a total of 21 out of 29 days resulted in no observed behavioral disturbances (Hoechst 1984c). In addition, routine gross and histopathologic examination of the cerebrum, cerebellum, brain stem, optic nerve, and pituitary demonstrated no treatment-related abnormalities.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to endosulfan.

No studies were located that examined reproductive function in animals after inhalation exposure to endosulfan. However, routine gross and histopathological examination of the reproductive organs (testes, epididymides, seminal vesicles, prostate, ovaries, and uterus) of rats exposed (nose-only) to concentrations of endosulfan of up to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days revealed no adverse effects (Hoechst 1984c).

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following inhalation exposure to endosulfan.

2.2.1.7 Genotoxic Effects

DNA damage in mononuclear leukocytes, as measured with the alkaline comet assay, was significantly increased in two of four French agricultural workers on the day following the application of pesticide mixtures, including endosulfan, compared to levels of DNA damage prior to application (Lebailly et al. 1998). However, the contribution of endosulfan to the observed effect is uncertain because of co-exposure to fungicides, herbicides, and other insecticides. Evaluations for micronuclei in human peripheral blood lymphocytes provided mixed results, depending on the analytical method used. No increase over control levels was observed in the frequency of micronuclei in peripheral blood lymphocytes of Chilean pesticide sprayers, using the cytochalasin-B method of arresting cytokinesis (Venegas et al. 1998), although endosulfan was reportedly applied by the workers only 3.7% of the time. In Italian greenhouse workers who applied a variety of pesticides including endosulfan, the frequency of micronuclei was increased compared to controls in an assay that used the 5-bromodeoxyuridine DNA-labeling technique (Falck et al. 1999), but not in an assay utilizing cytochalasin-B to arrest cytokinesis

(Scarpato et al. 1996a, 1996b). Previous studies in the same greenhouse worker group also showed no increase in chromosome aberrations or sister chromatid exchanges (Scarpato et al. 1996a, 1996b, 1997). The results of all of these genotoxicity studies in humans should be treated with caution because the multiple-chemical exposures confound the interpretation, and exposure levels of endosulfan were not reported.

Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding cancer in animals after inhalation exposure to endosulfan.

In a case-control study of the relation between occupational exposures to various suspected estrogenic chemicals and the occurrence of breast cancer, the breast cancer odds ratio (OR) was not elevated above unity (OR=0.8; 95% CI=0.2–3.2) for occupational exposure to endosulfan compared to unexposed controls (Aschengrau et al. 1998); however, the sample sizes were very small (three exposed; seven not exposed), and co-exposure to other unreported chemicals also reportedly occurred. Both of these factors may have contributed to the high degree of uncertainty in the OR indicated by the wide confidence interval.

2.2.2 Oral Exposure

2.2.2.1 Death

Acute accidental or intentional ingestion of large amounts of endosulfan has resulted in death in humans. Five cases of acute lethal poisoning in humans resulting from ingestion of Thiodan® were reported by Terziev et al. (1974). In two cases of suicide, the ingested dose was reported to be up to 100 mL of Thiodan® (concentration of endosulfan in this particular formulation was not specified); in the other three poisonings, the victims drank liquids containing the pesticide, but the ingested doses were not specified. Initial clinical symptoms of endosulfan poisoning included gagging, vomiting, diarrhea, agitation, writhing, loss of consciousness, cyanosis, dyspnea, foaming at the mouth, and noisy breathing. Autopsies performed in three out of five cases revealed edema of the brain and lungs, hemorrhage of the medullary layer of the kidneys, acute lung emphysema, and chromatolysis of the neurons. Two cases of lethal ingestion of endosulfan-containing formulations were reported by Demeter and Heyndrickx (1978). In

one, a 40-year-old man who consumed Posidor (20% endosulfan and 30% dimethoate in xylene) and alcohol died within 3 hours. His body was dark-red/purple, and his face was cyanotic. Autopsy revealed edematous lungs. The authors suggested that death was due to the combined effects of dimethoate (an organophosphate insecticide compound and potent cholinesterase inhibitor) and endosulfan. In the other case, a 28-year-old man ingested a fatal dose of Thiodan® powder (20% endosulfan) in conjunction with alcohol. Postmortem findings included congested and edematous lungs. Death was due to asphyxiation, which the authors suggested was caused by a synergistic effect of alcohol and endosulfan. In neither case was the ingested dose of endosulfan quantified. In the case of a 55-year-old female who died following intentional ingestion of an unspecified amount of endosulfan dispersed in a colorless liquid containing 55% xylene, autopsy revealed no gross anatomical or histological abnormalities attributed to endosulfan (Bernardelli and Gennari 1987). The presence of malignant melanoma and the concurrent ingestion of xylene may have been contributing factors in the death of this woman.

A more recent lethal case of a woman who ingested an unknown amount of endosulfan mistakenly added to food was reported (Blanco-Coronado et al. 1992). One to four hours after ingestion she had tonic-clonic convulsions, nausea, vomiting, headache, and dizziness. On admission to the hospital, the concentration of endosulfan (both isomers) in the gastric contents, blood, and urine was 55.4, 2.9, and 3 mg/L, respectively. She died 8 days after admission to the hospital following acute renal failure, disseminated intravascular coagulation, thrombi in the pulmonary arteries and aorta, and cardiogenic shock. Postmortem finding included bilateral pleural effusions, congested and edematous lungs with exudative areas and pulmonary edema, hyaline membranes, microatelectasia, polymorphonuclear lymphocytes and red cells in the alveoli, and interstitial fibrosis. A similar lethal case of a man who died 10 days after ingesting an unknown amount of endosulfan was described by Lo et al. (1995). The cause of death was described as cardio-respiratory arrest and heart failure and pulmonary edema. None of these case reports provide sufficient data to estimate a lethal dose of endosulfan in humans.

An estimated oral dose of 260 mg endosulfan/kg caused severe seizures in a 43-year-old man, and brain death from cerebral herniation and massive cerebral edema occurred within 4 days of exposure (Boereboom et al. 1998); there were no signs of myocardial infarction and only slight congestion of the heart, but pulmonary congestion and atelectasis were evident at autopsy.

Signs of acute lethal endosulfan poisoning in animals are similar to those observed in humans and include hyperexcitability, dyspnea, decreased respiration, and fine tremors followed by tonic-clonic convulsions. Oral LD_{50} (lethal dose, 50% kill) values for technical-grade endosulfan vary depending on species, sex,

formulation tested, and nutritional status of the animal (Gupta and Gupta 1979; WHO 1984). With regard to species sensitivity, mice appear to be quite sensitive to endosulfan's lethal effects, with a reported LD_{50} value of 7.36 mg/kg in males (Gupta et al. 1981) and 2 out of 10 male mice dying after administration of 7.5 mg technical endosulfan/kg in the diet for 7 days (Wilson and LeBlanc 1998). In contrast, LD_{50} values in male rats range between 40 and 121 mg/kg (Boyd and Dobos 1969; Boyd et al. 1970; Hoechst 1990; Lindquist and Dahm 1957). A single oral dose of 20 mg/kg of technical endosulfan killed 3 out of 14 male rats within hours of dosing, following seizure activity (Gilbert and Mack 1995). Six of 19 male rats died during the first 2 weeks of gavage treatment with 7.5 mg technical endosulfan/kg/day (Ansari et al. 1984). An LD_{50} value of 76.7 mg/kg has been calculated from the results of one dog study (sex and breed not indicated) (Hoechst 1970). However, endosulfan causes vomiting in dogs, and one study found that all dogs died that did not vomit after ingesting doses of at least 30 mg/kg (FMC 1958). Thus, the value of the dog LD_{50} may reflect both the dose and whether or not the dogs vomited. The acute toxicity of endosulfan also has been tested in rabbits, a species in which a single gavage dose of 15.1 mg of technical endosulfan/kg killed five out of seven animals (Ceron et al. 1995). Three of these died 15–20 minutes following dosing after experiencing clonic-tonic convulsions and fine generalized tremors. The other two showed similar signs and died 2–3 hours after dosing.

In rats, exposed males and females appear to have different sensitivities to the lethal effects of endosulfan exposure. Summary data submitted by Hoechst (1990) showed that female LD_{50} values ranged between 10 and 23 mg/kg, whereas male LD_{50} values ranged between 40 and 125 mg/kg. Thus, female rats appear to be 4–5 times more sensitive to the lethal effects of technical-grade endosulfan than male rats. This difference may be related to differences in the toxicokinetics of endosulfan in male and female rats (see also Section 2.3). Insufficient data were available to determine whether differences in sensitivity to lethal effects exist between males and females of species other than the rat.

The effects of protein deficiency on endosulfan toxicity were studied in Wistar rats (Boyd and Dobos 1969; Boyd et al. 1970). Rats fed a diet totally deficient in protein for 28 days prior to administration of a single oral dose of endosulfan had an LD_{50} of 5.1 mg/kg of endosulfan. Rats fed a low-protein diet (3.5% protein) for 28 days had an LD_{50} of 24 mg/kg of endosulfan. Rats fed standard laboratory chow (26% protein) had an LD_{50} of 102–121 mg/kg. The immediate cause of death in all animals was respiratory failure following tonic-clonic convulsions. This study demonstrated that, while a protein-deficient diet does not affect the nature of the toxic reaction, it may affect the sensitivity of rats to the lethal effects of endosulfan.

The two isomers of endosulfan (α-and β-) also have different LD_{50} values in rats. The α-isomer is more toxic than the β-isomer in female rats, with an oral LD_{50} value of 76 mg/kg versus an LD_{50} value of 240 mg/kg for β-endosulfan (Hoechst 1975, 1990; Maier-Bode 1968). The same difference was reported in female albino mice, the lethal dose for α-endosulfan being 11 mg/kg versus 36 mg/kg for β-endosulfan (Dorough et al. 1978). The lethal dose for endosulfan sulfate in mice was comparable to that of the α-isomer, 8 mg/kg (Dorough et al. 1978). Also, Hoechst (1966a, 1966b) had reported an LD_{50} of 14 mg/kg for α-endosulfan and 17 mg/kg for β-endosulfan in female mice.

In rats, daily administration of 5 or 10 mg/kg doses of endosulfan by gavage in corn oil during gestational days (Gd) 6–14 or 6–19 produced a dose-related increase in maternal deaths in these test groups (FMC 1980a; Gupta et al. 1978).

In intermediate-duration studies, rats tolerated 6-day/week gavage doses of 20 mg/kg endosulfan for 7 weeks (Garg et al.1980), whereas increased mortality was observed in male and female mice at doses of 7.3 mg/kg/day and 7.52 mg/kg/day, respectively, for 13 weeks (Hoechst 1984b). Two out of 19 rats administered 7.5 mg technical endosulfan/kg/day died in a 60-day study (Ansari et al. 1984). A more recent study also found female rats to be more sensitive than males, since 3 out 10 females died during a 30-day feeding study, but no deaths occurred in male groups (Paul et al. 1995). An additional intermediate-duration study reported that 4 out of 15 male rats (females not tested) died after administration of 10 mg technical endosulfan/kg, 3 times a week for 4–5 weeks (Gilbert 1992). Male and female dogs orally administered time-weighted average (TWA) doses of 2.9 and 2.6 mg/kg/day, respectively, were sacrificed at the end of 146 days because of severe neurological symptoms (Hoechst 1989c).

Increased mortality was observed in both male rats (at doses of 20.4 mg/kg/day and above) and male mice (at doses of 0.46 mg/kg/day and above) in a 2-year bioassay conducted by the National Cancer Institute (NCI 1978). The authors attributed the excessive mortality in the male rats to treatment-related toxic nephropathy. The high mortality in male mice was possibly due to fighting since no other treatment-related cause for the deaths could be determined. Survival in females of both species was unaffected by endosulfan (NCI 1978). However, survival was significantly decreased in female rats that consumed 5 mg/kg/day for 2 years (FMC 1959b), and in female mice that consumed approximately 2.9 mg technical endosulfan/kg/day for 2 years (Hack et al. 1995; Hoechst 1988b). In these studies, survival in male rats was not affected at 5 mg/kg/day for 2 years (FMC 1959b) and survival in male mice was not affected at 2.51 mg/kg/day for 2 years (Hoechst 1988b).

All reliable LD_{50} and LOAEL values for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

2.2.2.2 Systemic Effects

Case reports of human poisonings and studies in animals indicate that during acute oral exposure to lethal or near-lethal amounts of endosulfan, involvement of a large number of organ systems (respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal) is observed. However, during longer-term exposure, the liver and kidney appear to be the primary systemic target organs.

The highest NOAEL values and all reliable LOAEL values for systemic effects for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

Respiratory Effects. Respiratory effects have been observed in cases of lethal poisonings from intentional or accidental ingestion of large quantities of endosulfan. Cyanosis, dyspnea, foaming at the mouth, and noisy breathing have been observed in several subjects prior to death (Blanco-Coronado et al. 1992; Terziev et al. 1974). At autopsy, acute emphysema and/or congested and edematous lungs were frequently observed in persons following ingestion of lethal quantities of endosulfan (Blanco-Coronado et al. 1992; Boereboom et al. 1998; Demeter and Heyndrickx 1978; Lo et al. 1995; Terziev et al. 1974). Respiratory effects were also part of the clinical syndrome displayed by a 20-year-old man who attempted suicide by ingesting 200 mL of a 30% endosulfan formulation (Thionax®) (Shemesh et al. 1988). Although the man's stomach was pumped and he was given activated charcoal to limit gastrointestinal absorption during the first 16 hours following ingestion, hypoxia (due to alveolar hypoventilation and pulmonary edema) was evident. In the following 2 weeks, the patient had recurrent aspiration pneumonia and a persistent need for mechanical ventilation. Although it is possible that these respiratory effects were due, in part, to a direct action of endosulfan on the lungs, it is more likely that many of the observed effects were secondary to endosulfan's direct effects on the central nervous system and its control of respiratory activity. It is unclear whether other ingredients in the Thionax® contributed to the effects observed. Respiratory effects were also reported in other nonlethal cases of acute intoxication with endosulfan. Pulmonary infiltrate was reported in 3 out of 4 cases 4 hours after ingesting an unknown amount of endosulfan, and 4 of these 5 cases required mechanical ventilation (Blanco-Coronado et al.

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Human	once				260 M (lethal dose)	Boereboom et al. 1998 Technical
2	Rat (albino)	60 d 1x/d (GO)				7.5 M (6/19 died; 2 on day 3 and 4 on day 14)	Ansari et al. 1984 Technical
3	Rat (Wistar)	once (GO)				24 M (LD_{50}; low protein diet)	Boyd and Dobos 1969 Technical
4	Rat (Sprague-Dawley)	Gd 6-19 2 wk 1x/d (G)				6 F (2/25 died)	FMC 1980b Technical
5	Rat (Long- Evans)	once (GO)				20 M (3 of 14 died following seizure activity)	Gilbert and Mack 1995 Technical
6	Rat (albino)	Gd 6-14 9 d 1x/d (GO)				10 F (5/32 died)	Gupta et al. 1978 Technical
7	Rat (Wistar)	once (GW)				240 F (LD_{50})	Hoechst 1975 Beta
8	Rat (Wistar)	once (GW)				65.7 F (LD_{50})	Hoechst 1988a Beta

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
9	Rat (Wistar)	once (GW)				1740 M	(LD_{50})	Hoechst 1988a Beta
10	Mouse (albino)	once (GW)				11 F	(lethal dose)	Dorough et al. 1978 Alpha
11	Mouse (albino)	once (GW)				36 F	(lethal dose)	Dorough et al. 1978 Beta
12	Mouse (albino)	once (GW)				8 F	(lethal dose)	Dorough et al. 1978 endosulfan sulfate
13	Mouse (albino)	NS (GO)				7.4 M	(LD_{50})	Gupta et al. 1981 Technical
14	Mouse	once (GW)				14 F	(LD_{50})	Hoechst 1966a Alpha
15	Mouse	once (GW)				17 F	(LD_{50})	Hoechst 1966b Beta
16	Mouse (CD-1)	7 d (F)				7.5 M	(2 of 10 died)	Wilson and LeBlanc 1998 Technical
17	Dog	once (C)				77	(LD_{50})	Hoechst 1970 Technical
18	Rabbit (New Zealand)	once (GW)				15.1 M	(5 of 7 died following seizure activity)	Ceron et al. 1995 Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
19	Human	once	Resp			260 M (pulmonary congestion, atelectasis)	Boereboom et al. 1998 Technical
			Cardio			260 M (blood pressure drop)	
			Gastro	260 M			
			Hemato		260 M (elevated hemoglobin and white cell count)		
			Hepatic		260 M (liver congestion, fatty degeneration)		
			Renal	260 M			
20	Rat (Charles Foster)	1 or 5 d 1x/d (G)	Endocr		5 (degranulation of beta-cells of islets of Langerhans)		Barooah et al. 1980 Technical
			Metab		5 (decreased serum glucose)		
21	Rat (albino)	once (GO)	Metab		40 M (increased blood glucose)		Garg et al. 1980 Technical
22	Rat (Wistar)	once (GW)	Resp	63 F		70 F (lungs congestion)	Hoechst 1988a Beta
			Gastro			63 F (blood in small intestines; mucus in stomach)	

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
23	Rat (Kasauli)	4 d 1x/d (GO)	Hemato		12.5 F	(decreased erythrocyte [Na+, K+]--ATPase activity)			Kiran and Varma 1988 Technical
			Hepatic		12.5 F	(decreased liver aldolase)			
			Metab		12.5 F	(increased blood glucose; decreased liver glycogen)			
24	Rat (NS)	1 d 1x/d (G)	Resp				200 M	(dyspnea, emphysema, lung hemorrhages; cyanosis)	Terziev et al. 1974 Technical
			Cardio				200 M	(myocardial hemorrhages)	
			Renal				200 M	(hemorrhages in the kidney)	
25	Dog (Mongrel)	3 d 1x/d (C)	Gastro		2.5	(vomiting)			FMC 1959a Technical
26	Dog	once (C)	Resp				50	(respiratory paralysis; congestion of the lungs)	Hoechst 1970 Technical
			Gastro		50	(congestion in the stomach and small intestine)			
			Hepatic		50	(congestion of the liver)			
			Renal		50	(congestion of the kidneys)			

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
27	Rabbit (New Zealand)	once (GW)	Gastro		15.1 M (watery diarrhea)		Ceron et al. 1995 Technical
			Hemato		15.1 M (reduced red blood cell counts, packed cell volume and hemoglobin)		
			Hepatic		15.1 M (increased serum AP, AST, and ALT activities)		
			Bd Wt		15.1 M (12% body weight loss)		
			Other		15.1 M (82% reduction in food intake)		
	Immunological/Lymphoreticular						
28	Rat (Wistar)	10 d (GO)		4.5 F			Hoechst 1988c Technical
	Neurological						
29	Human	once				260 M (convulsions, cerebral edema, cerebral herniation, sustained epileptic state)	Boereboom et al. 1998 Technical
30	Rat (Sprague-Dawley)	Gd 6-19 14 d (GO)				2.5 F (poor muscle tone;head swaying)	FMC 1980a Technical
31	Rat (Long- Evans)	once (GO)				5 M (seizures)	Gilbert and Mack 1995 Technical
32	Rat (Wistar)	once (GW)				80 F (hyperactivity; convulsions; tremors)	Hoechst 1984e Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
33	Rat (Wistar)	once (GW)					63 F (clonic spasms)	Hoechst 1988a Beta
34	Rat (Kasauli)	4 d 1x/d (GO)					12.5 F (tremors)	Kiran and Varma 1988 Technical
35	Rat (Wistar)	8 d 1 x/d (GO)			6	(changes in transmitter levels in several brain areas)		Lakshmana and Raju 1994 Technical
36	Rat (NS)	once (G)					200 M (brain edema, convulsions)	Terziev et al. 1974 Technical
37	Mouse	once (GW)					10 F (convulsions)	Hoechst 1966a Alpha
38	Mouse	once (GW)					12.5 F (convulsions)	Hoechst 1966b Beta
39	Dog	3 d 1x/d (C)					2.5 (convulsions; salivation; tremors; rapid respiration; pupillary dilation)	FMC 1959a Technical
40	Dog	once (C)		39.5			50 (convulsions, respiratory paralysis)	Hoechst 1970 Technical
41	Rabbit (New Zealand)	10 d 1x/d (GO)		0.7			1.8 F (tachypnea; hyperactivity; convulsions in dams)	FMC 1981 Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Reproductive						
42	Rat (Sprague-Dawley)	Gd 6-19 2 wk 1x/d (G)		6 F			FMC 1980b Technical
	Developmental						
43	Rat (Sprague-Dawley)	Gd 6-19 2 wk 1x/d (G)			6 (increased skeletal variations; decreased birth weight and length)		FMC 1980b Technical
44	Rat (albino)	Gd 6-14 9 d 1x/d (GO)				5 (increased resorptions and skeletal variations)	Gupta et al. 1978 Technical
	INTERMEDIATE EXPOSURE						
	Death						
45	Rat (albino)	60 d 1x/d (GO)				7.5 M (2/19 died on days 32 and 58)	Ansari et al. 1984 Technical
46	Rat (Long- Evans)	7 wk 3 x/wk (GO)				10 M (4/16 died following 4-5 weeks of dosing)	Gilbert 1992 Technical
47	Rat (Wistar)	30 d (F)				6 F (3 out 10 died)	Paul et al. 1995 Technical
48	Mouse (CD-1)	13 wk ad lib (F)				7.3 M (10/20 died) 7.5 F (11/20 died)	Hoechst 1984b Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
49	Rat (albino)	9-18 wk ad lib (F)	Hemato	5			Das and Garg 1981 Technical
			Hepatic	5			
			Renal	5			
			Bd Wt	5			
50	Rat (albino)	30 d 1x/d (GO)	Hemato	1.5 F	5 M (increased RBC and neutrophil count)		Dikshith et al. 1984 Technical
			Hepatic		1.5 F (increased liver alkaline phosphatase)		
				1.5	5 M (increased relative liver weight)		
			Renal	1.5			
			Bd Wt	1.5			
51	Rat (Wistar)	26 wk ad lib (F)	Hemato	5			FMC 1959b Technical
52	Rat (albino)	7 wk 6 d/wk 1x/day (GO)	Metab	0.625 M	5 M (decreased blood calcium)		Garg et al. 1980 Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
53	Rat (albino)	15 d 1x/d (GO)	Resp	5 M	10 M (inflammation of lungs; dilation of alveoli)	10 M (more severe necrosis; inflammation, dilation, and congestion of central veins and sinusoids)	Gupta and Chandra 1977 Technical
			Gastro	10 M			
			Hepatic		5 M (increased absolute and relative liver weight; dilation of sinusoids; necrosis)		
			Renal	5 M		10 M (congestion and degeneration of kidney tubules)	
			Endocr	10 M			
			Bd Wt			10 M (30% less weight gain than controls)	
54	Rat (albino)	15 d 1x/d (GO)	Hepatic	5.0 F			Gupta and Gupta 1977a Technical
			Endocr	5.0 F			
			Bd Wt	5.0 F			
55	Rat (CrL:COBS CD)	84 d ad lib (F)	Bd Wt	0.8	3.8 (decreased body weight gain in dams)		Hoechst 1984a Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
56	Rat (Sprague-Dawley)	13 wk ad lib (F)	Hemato		0.8 (decreased hemoglobin)		Hoechst 1985a Technical
			Hepatic	3.8 M	23.4 M (granular brown pigment; increased liver weight)		
				3.8 F	23.4 F (increased liver weight; centrilobular enlargement, increased serum lipids and cholesterol)		
			Renal	1.9	3.9 (yellow protein in tubule lumen; eosinophilic droplets in cells of proximal convoluted tubules; increased kidney weights)	23.4 M (proteinuria)	
			Dermal	2.3	4.6 F (hair loss)		
57	Rat (Sprague-Dawley)	13-26 wk ad lib (F)	Hemato	2.9			Hoechst 1989a Technical
58	Rat (Wistar)	30 d (F)	Hepatic		3 F (increased serum and liver AST, ALT and AP activities)		Paul et al. 1995 Technical
					6 M (increased liver AST, ALT and liver and serum AP activities)		
			Bd Wt	6			
59	Rat (albino)	30 d 1x/d (GO)	Resp		1.5 F (transient dyspnea)		Raizada et al. 1991
			Endocr	1.5 F			

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
60	Rat (Wistar)	15-30 d 1x/d (GO)	Endocr		7.5 M	(reduced testicular and plasma hormones, decreased enzyme activity levels)			Singh and Pandey 1990 Technical
61	Rat (Druckrey)	70 d 5 d/wk (GO)	Bd Wt	10 M					Sinha et al. 1995 Technical
62	Mouse (CD-1)	13 wk ad lib (F)	Resp	2.1			7.3	(vascular congestion in lungs)	Hoechst 1984b Technical
			Cardio	7.3					
			Gastro	7.3					
			Hemato	7.3					
			Musc/skel	7.3					
			Hepatic	7.3					
			Renal	7.3					
			Endocr	7.3					
			Ocular	7.3					
63	Mouse (NMRI)	42 d ad lib (F)	Ocular	3.7					Hoechst 1985b Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
64	Dog (Beagle)	146-147 d ad lib (F)	Resp	2.6			Hoechst 1989c Technical
			Cardio	2.6			
			Gastro	2.6			
			Hemato	2.6			
			Musc/skel	2.6			
			Hepatic		2.6 (elevated serum alkaline phosphatase)		
			Renal	2.6			
			Endocr	2.6			
			Dermal	2.6			
			Ocular	2.6			
65	Rabbit (New Zealand)	Gd 6-28 23 d 1x/d (G)	Resp		1.8 F (noisy and rapid breathing)		FMC 1981 Technical
	Immunological/Lymphoreticular						
66	Rat (Wistar)	8-22 wk ad lib (F)		0.45[b] M	0.9 M (decreased humoral and cell-mediated response)		Banerjee and Hussain 1986 Technical
67	Rat (Wistar)	6 wk ad lib (F)		0.9 M	2.7 M (decrease in humoral antibody and cell-mediated immune response)		Banerjee and Hussain 1987 Technical
68	Rat (Wistar)	3 wk ad lib (F)		5 M			Vos et al. 1982 Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
69	Mouse (CD-1)	13 wk ad lib (F)		2.1 M	7.3 M (decreased neutrophils and relative spleen weight)		Hoechst 1984b Technical
	Neurological						
70	Rat (NS)	60 d 1x/d (GO)		2.5 M		7.5 M (hyperactivity; tremors; convulsions)	Ansari et al. 1984 Technical
71	Rat (albino)	30 d 1x/d (GO)				1.5 (hyperexcitation and tremors)	Dikshith et al. 1984 Technical
72	Rat (Long- Evans)	20 d 1 x/d (GO)				5 M (increased seizure activity)	Gilbert 1992 Technical
73	Rat (CrL:COBS CD)	84 d (F)		0.8	3.8 F (increased brain weight in dams)		Hoechst 1984a Technical
74	Rat (Sprague-Dawley)	13 wk ad lib (F)		2.3	4.6 F (increased brain weight)		Hoechst 1985a Technical
75	Rat (Wistar)	23 d 1 x/d (GO)			6 (changes in transmitter levels in several brain areas; impaired learning of a task)		Lakshmana and Raju 1994 Technical
76	Rat (Wistar)	90 d 1 x/d (GW)				2 M (inhibition of learning and memory processes)	Paul et al. 1994 Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
77	Rat (Wistar)	30 d (F)				3 (impaired learning and memory processes)	Paul et al. 1995 Technical
78	Rat (albino)	30 d 1x/d (GO)			1.5 F (transient hyperactivity, tremor, salivation)		Raizada et al. 1991
79	Mouse (CD-1)	13 wk ad lib (F)				7.3 (convulsions)	Hoechst 1984b Technical
80	Dog (Beagle)	146-147 d 1x/d (F)				2.6 (extreme sensitivity to noise and optical stimuli; muscle spasms in extremeties, face, and jaw; placing and righting reflexes absent)	Hoechst 1989c Technical
	Reproductive						
81	Rat (Sprague-Dawley)	170+ d ad lib (F)		2.5			FMC 1965 Technical
82	Rat (albino)	15 d 1x/d (GO)		5 M		10 M (degeneration of seminiferous tubule epithelium)	Gupta and Chandra 1977 Technical
83	Rat (Sprague-Dawley)	11 wk ad lib (F)		8			Hoechst 1982 Technical
84	Rat (CrL:COBS CD)	84 d (F)		3.8			Hoechst 1984a Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
85	Rat (Druckrey)	70 d 5 d/wk (GO)				2.5 M (reduced sperm count; changes in enzyme activity indicating altered spermatogenesis)	Sinha et al. 1995 Technical
86	Rat (Druckrey)	90 d 5 d/wk (GO)				2.5 M (reduced sperm and spermatids count and daily sperm production; increased abnormal sperm; changes in enzyme activity indicating altered spermatogenesis)	Sinha et al. 1997 Technical
87	Mouse (Swiss albino)	35 d 1x/d (GW)				3 M (4/5 reduction in sperm count; increased frequency of sperm with head abnormalities)	Khan and Sinha 1996 Technical
88	Rabbit (New Zealand)	Gd 6-28 23 d 1x/d (G)		1.8 F			FMC 1981 Technical
	Developmental						
89	Rat (Sprague-Dawley)	11 wk ad lib (F)		4	6 (decreased mean litter weights during lactation)	8 (increased pup mortality post-weaning)	Hoechst 1982 Technical
90	Rat	84 d (F)		0.2	0.8 (decreased litter weights prior to weaning)		Hoechst 1984a Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
91	Rabbit (New Zealand)	Gd 6-28 23 d 1x/d (GO)		1.8			FMC 1981 Technical
	CHRONIC EXPOSURE						
	Death						
92	Rat (Wistar)	2 yr ad lib (F)				5.0 F (decreased survival)	FMC 1959b Technical
93	Mouse (NMRI)	104 wk (F)				2.88 F (28% survival compared to 45% in controls)	Hack et al. 1995 Technical
94	Mouse (NMRI)	24 mo ad lib (F)				2.9 F (decreased survival)	Hoechst 1988b Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
95	Rat (Wistar)	2 yr ad lib (F)	Resp	5.0			FMC 1959b Technical
			Cardio	5.0			
			Gastro	5.0			
			Hemato	5.0			
			Hepatic	1.0	5.0 M (hydropic hepatic cells)		
			Renal	1.0		5.0 M (increased kidney weight; renal tubule dilation, degeneration of renal tubule epithelium; albuminous casts; focal interstitial nephritis)	
			Endocr	5.0			

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
96	Rat (Sprague-Dawley)	104 wk (F)	Resp	3.5 F			Hack et al. 1995 Technical
			Cardio	3.5 F			
			Gastro	3.5 F			
			Hemato	3.5 F			
			Hepatic	3.5 F			
			Renal	0.7 F	3.0 M (greater number of aneurysms in kidneys, increased incidence of progressive glomeulonephrosis)		
					3.5 F (enlarged kidneys)		
			Ocular	3.5 F			
			Bd Wt		0.6 M (15% less weight gain)	3.0 M (29% less weight gain	
					0.7 F (11% less weight gain)	3.5 F (21% less weight gain)	
			Metab	3.5 F			
			Other	3.5 F			

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
97	Rat (Sprague-Dawley)	2 yr ad lib (F)	Resp	2.9			Hoechst 1989a Technical
			Cardio	0.6	2.9 M (aneurysms of blood vessels)		
			Gastro	2.9			
			Hemato	2.9			
			Musc/skel	2.9			
			Hepatic	2.9			
			Renal	0.6		2.9 M (severe glomerulonephrosis)	
			Endocr	2.0			
			Bd Wt	0.6	2.9 M (signif. reduced body wt gain)		
			Dermal	2.0			
			Ocular	2.0			
98	Rat (Osborne-Mendel)	74-82 wk ad lib (F)	Cardio			20 M (calcium deposits in the heart, coronary and mesenteric arteries)	NCI 1978 Technical
			Renal			11 (degeneration of proximal convoluted tubule; degeneration of tubular epithelium; fibrosis and focal mineralization)	
			Endocr		11 (parathyroid hyperplasia; calcification of organs)		
			Bd Wt			20 M (body weight reduced by 23% relative to controls at week 80)	

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
99	Mouse (NMRI)	104 wk (F)	Resp	2.88 F			Hack et al. 1995 Technical
			Cardio	2.88 F			
			Gastro	2.88 F			
			Hemato	2.88 F			
			Hepatic	2.88 F			
			Renal	2.88 F			
			Bd Wt	0.97 F	2.52 M (17% less weight gain)		
			Other	2.88 F			
100	Mouse (NMRI)	24 mo ad lib (F)	Resp	2.5			Hoechst 1988b Technical
			Cardio	2.5			
			Gastro	2.5			
			Hemato	2.5			
			Musc/skel	2.5			
			Hepatic	2.5			
			Renal	2.5			
			Endocr	2.5			
			Dermal	2.5			
			Ocular	2.5			
			Bd Wt	0.8	2.5 M (decreased body weight)		
101	Dog (Mongrel)	1 yr 6 d/wk 1x/d (C)	Resp	0.8			FMC 1959a Technical
			Cardio	0.8			
			Gastro	0.8			
			Hemato	0.8			
			Hepatic	0.8			
			Renal	0.8			
			Endocr	0.8			

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
102	Dog (Beagle)	2 yr ad lib (F)	Resp	1.0					FMC 1967 Technical
			Cardio	1.0					
			Gastro	1.0					
			Hemato	1.0					
			Musc/skel	1.0					
			Hepatic	1.0					
			Renal	1.0					
			Endocr	1.0					
103	Dog (Beagle)	1 yr ad lib (F)	Resp	1.8					Hoechst 1989c Technical
			Cardio	1.8					
			Gastro	0.6					
			Hemato	1.8					
			Musc/skel	0.6			1.8	(abdominal and jaw muscle spasms)	
			Hepatic	0.18[c]	0.6	(increased serum alkaline phosphatase)			
			Renal	1.8					
			Endocr	1.8					
			Dermal	1.8					
			Ocular	1.8					
	Immunological/Lymphoreticular								
104	Rat (Sprague-Dawley)	2 yr ad lib (F)		2.9					Hoechst 1989a Technical

Table 2-2. Levels of Significant Exposure to Endosulfan - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
	Neurological							
105	Rat (Sprague-Dawley)	2 yr ad lib (F)		2.9				Hoechst 1989a Technical
106	Mouse (NMRI)	24 mo ad lib (F)		2.5				Hoechst 1988b Technical
107	Dog (Beagle)	2 yr ad lib (F)		1.0				FMC 1967 Technical
108	Dog (Beagle)	1 yr ad lib (F)		0.6		1.8	(abdominal and jaw muscle spasms)	Hoechst 1989c Technical
	Reproductive							
109	Rat (Osborne-Mendel)	74-82 wk ad lib (F)		20 M		48 M	(testicular necrosis aspermatogenesis)	NCI 1978 Technical

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate oral minimal risk level (MRL) of 0.005 mg/kg/day; 0.45 mg/kg body weight dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[c]Used to derive a chronic oral minimal risk level (MRL) of 0.002 mg/kg/day; 0.18 mg/kg body weight dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; ALT = alanine amino transferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; ATPase = adenosine triphosphatase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = female (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; (GW) = gavage in water; Gd = gestation day; Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observable- adverse-effect level; NS = not specified; RBC = red blood cell; Resp = respiratory; wk = week(s); x = time(s); y = year(s).

Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral

Acute (≤14 days)

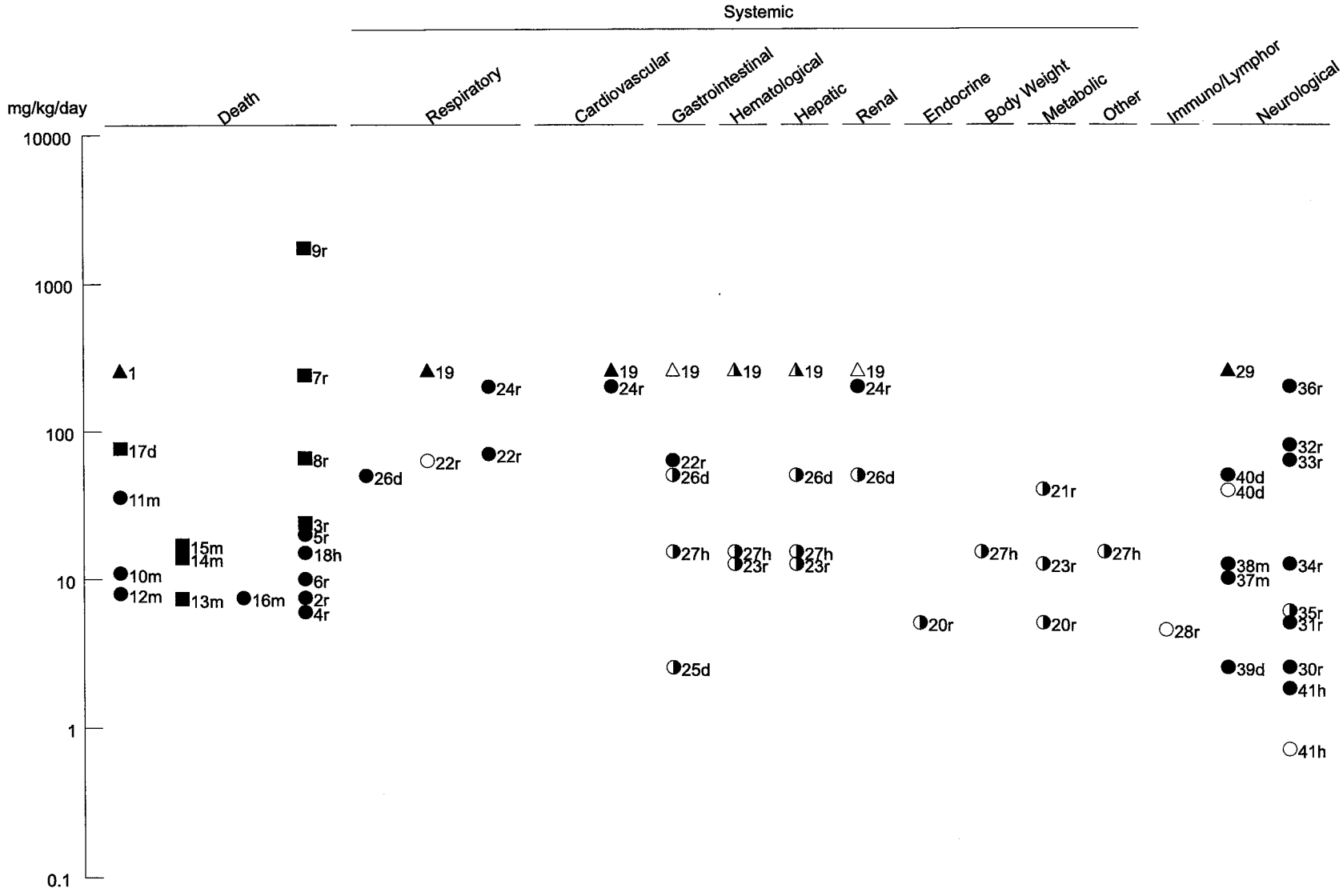


Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral (Continued)

Acute (≤14 days)

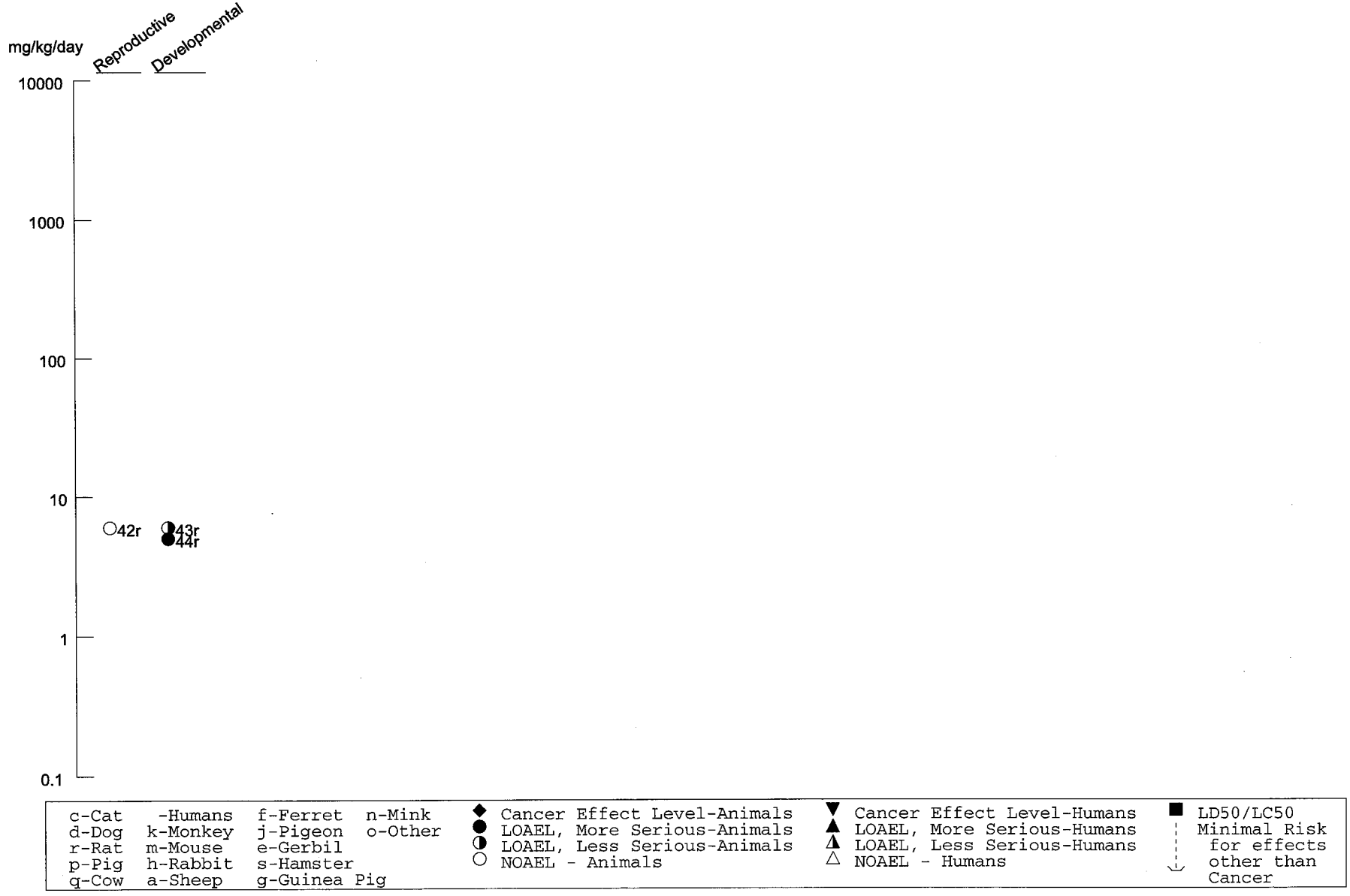


Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral (Continued)

Intermediate (15-364 days)

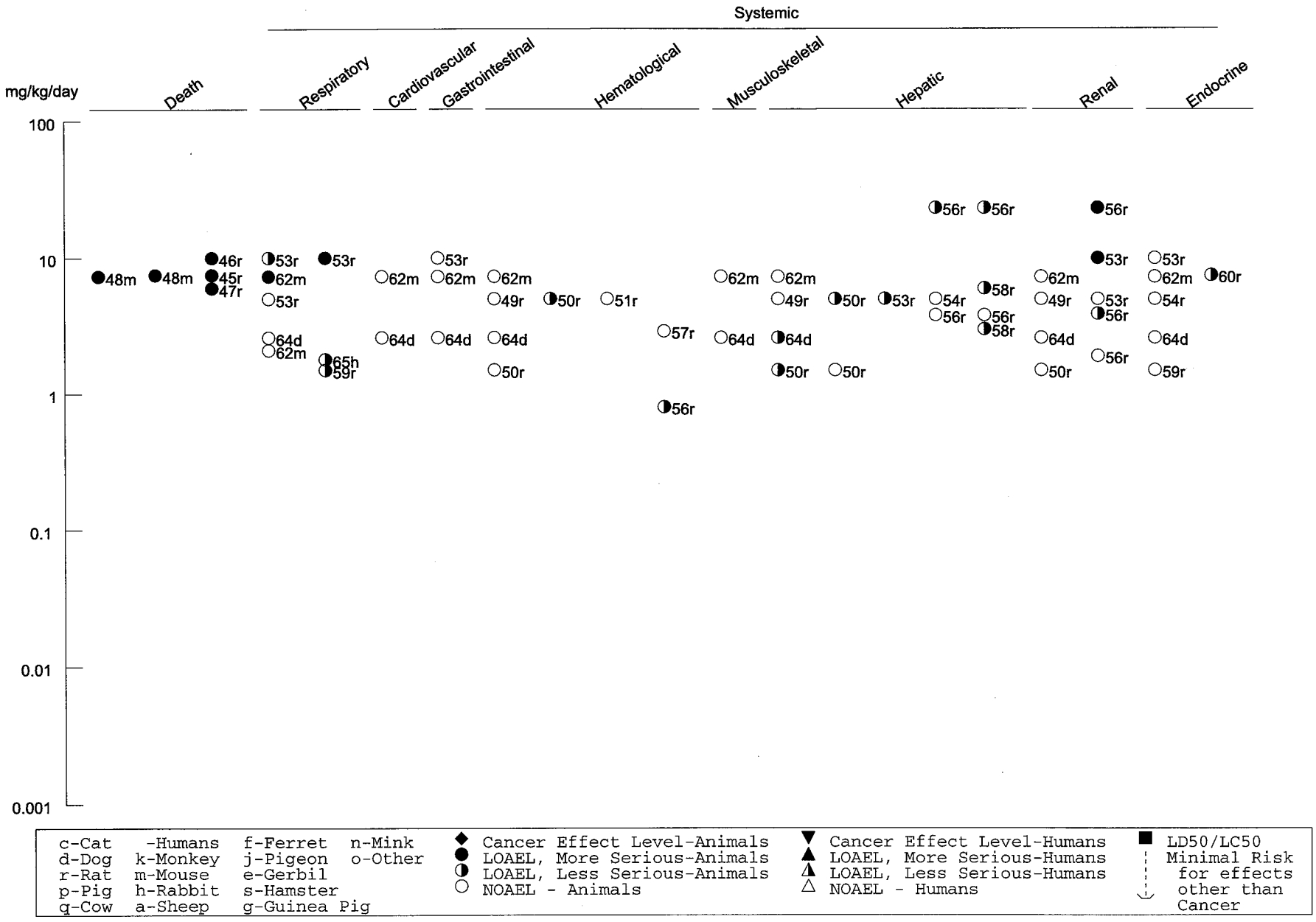


Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral (Continued)

Intermediate (15-364 days)

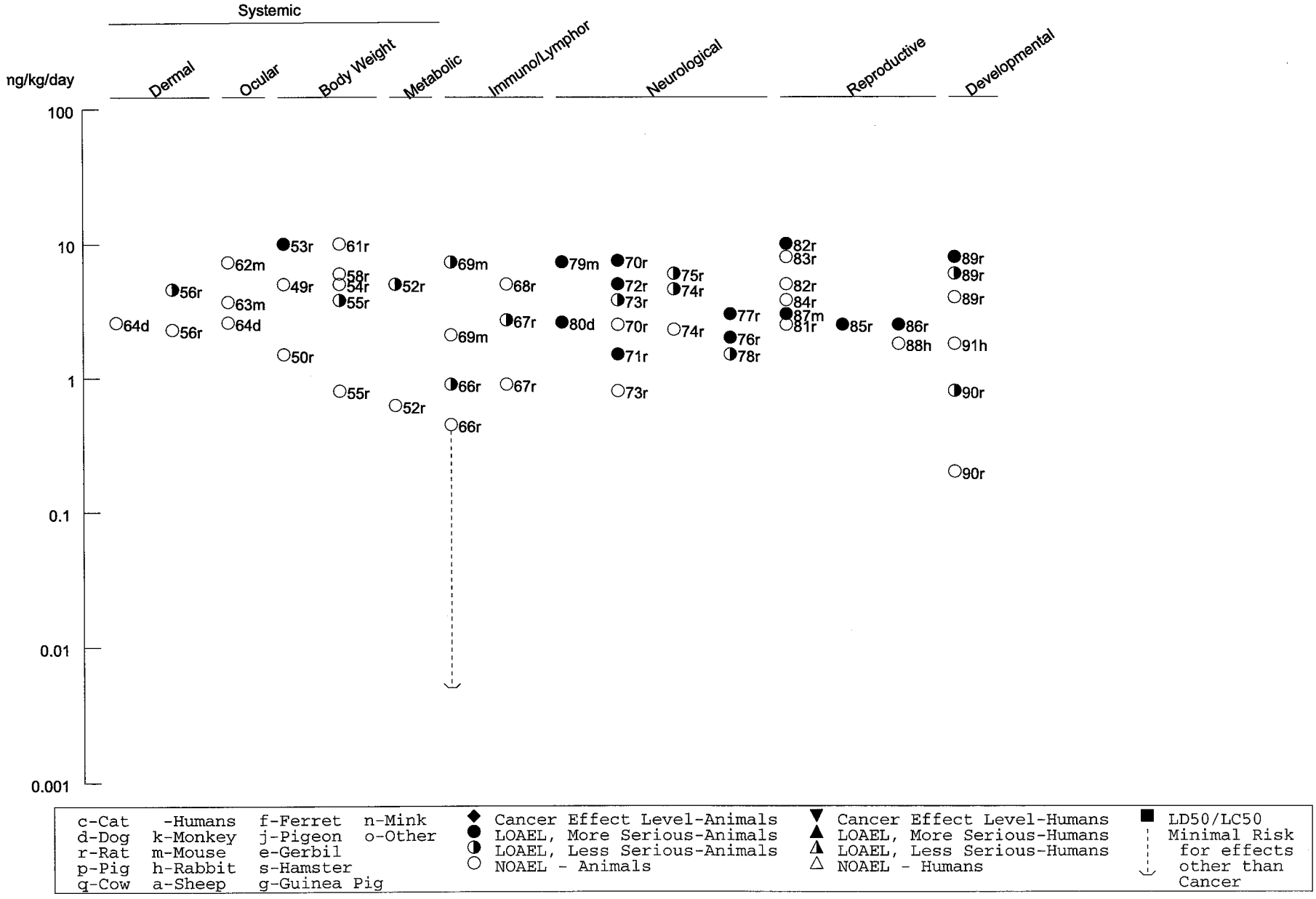


Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral (Continued)

Chronic (≥365 days)

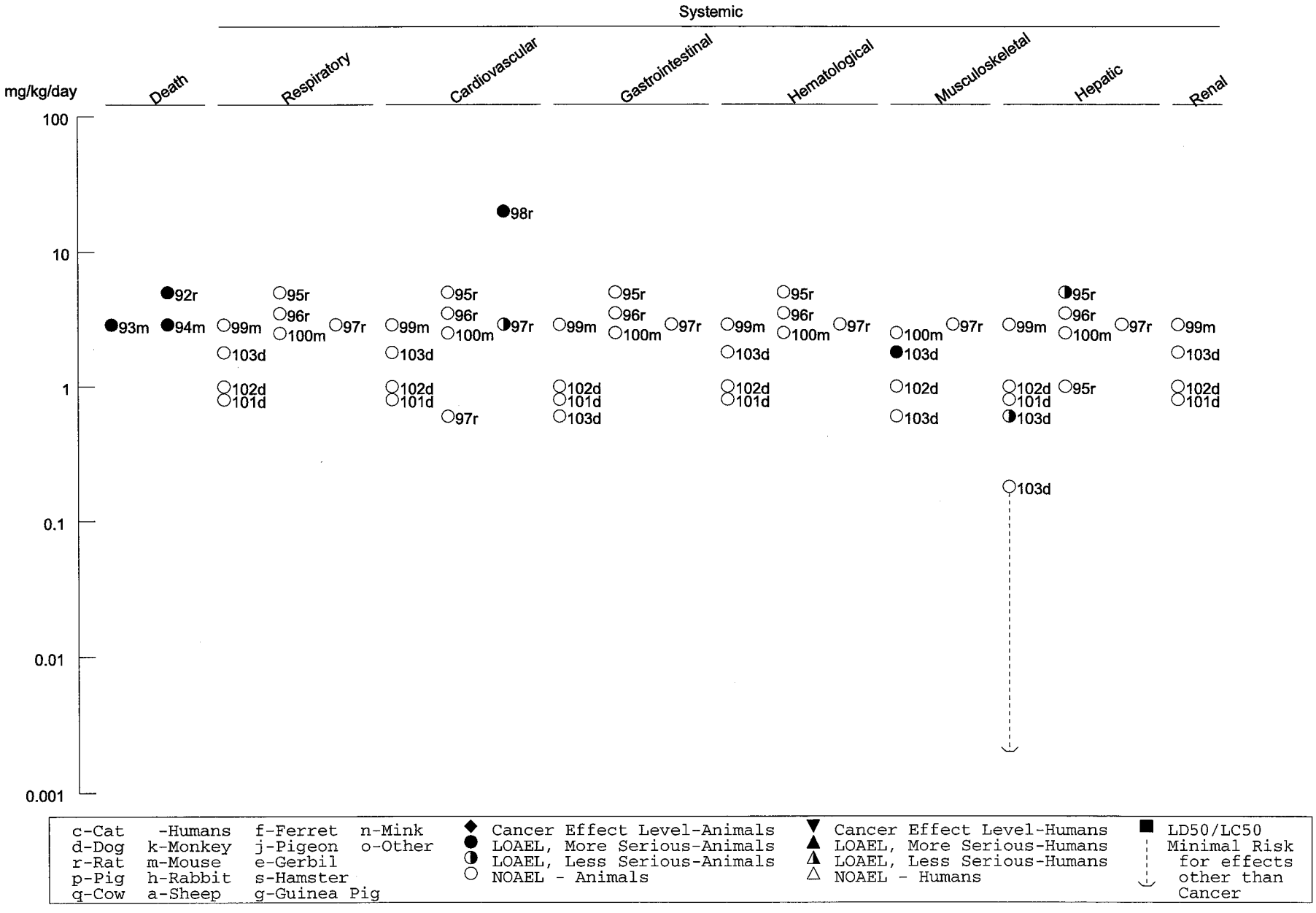
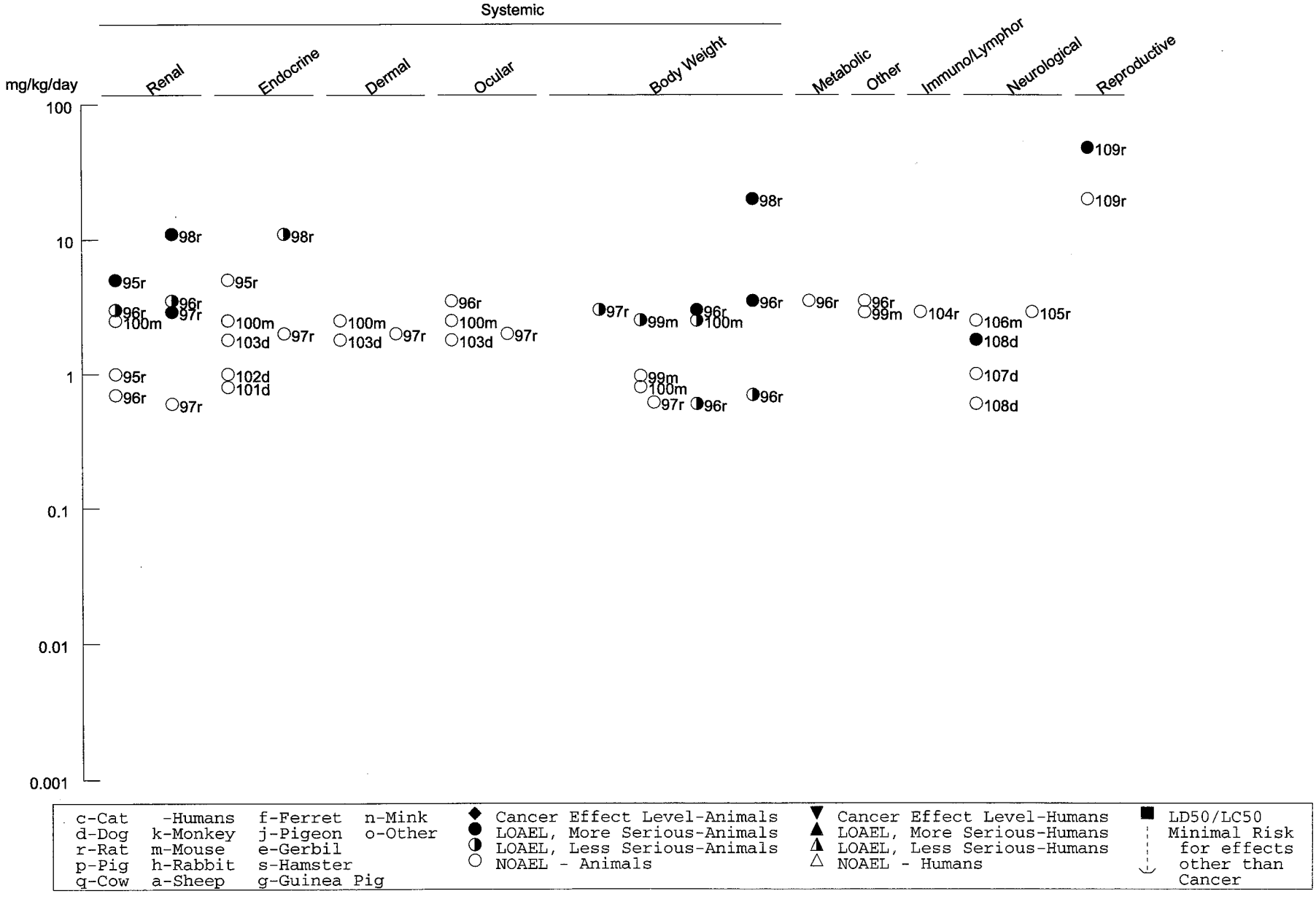


Figure 2-2. Levels of Significant Exposure to Endosulfan - Oral (Continued)

Chronic (≥365 days)



1992). Boereboom et al. (1998) observed minor lung hemorrhaging and atelectasis at autopsy of a man who ingested approximately 260 mg/kg endosulfan 4 days prior to death.

Similar to the data from humans, respiratory effects have been observed in animals almost exclusively in acute, lethal-dose exposure situations. Male rats given single gavage doses of 200 mg/kg of endosulfan exhibited dyspnea and cyanosis prior to death (Terziev et al. 1974). Necropsy revealed hemorrhages in the interalveolar partitions of the lung and acute emphysema of the lungs. Studies in which a limited number of dogs were given single oral doses of endosulfan as low as 10 mg/kg (FMC 1958) or 50 mg/kg (Hoechst 1970) demonstrated respiratory paralysis and death. Autopsy of the dogs revealed congestion of the lungs. It is not clear whether these effects were a result of direct action on the lungs or were associated with the generalized convulsions. In another study in which female rats were given a single gavage dose of β-endosulfan, lung congestion was observed at 70 mg/kg, but not at 63 mg/kg (Hoechst 1988a). These dose levels caused lethality in the rats.

Local inflammation of the lungs and dilated alveoli were observed in rats administered 10 mg/kg/day of endosulfan in peanut oil by gavage for 15 days (Gupta and Chandra 1977). However, there was high mortality in this dose group (3 of 8 animals died prior to study termination), and it is not clear if these effects were observed primarily in the intercurrent deaths or in animals surviving for the full 15 days of exposure.

With the exception of the effects reported by Hoechst (1988a) in female rats, no effects on respiratory tissues were observed during gross and histopathological examinations in intermediate- and chronic-duration studies in rats, mice, and dogs at sublethal doses (FMC 1959a, 1959b, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989a, 1989c).

Cardiovascular Effects. Cardiovascular effects were part of the clinical syndrome displayed by a 20-year-old man who attempted suicide by ingesting 200 mL of a 30% endosulfan formulation (Thionax®) (Shemesh et al. 1988). Although the man's stomach contents were aspirated and he was given activated charcoal to limit absorption during the first 16 hours following ingestion, episodes of tachycardia and hypertension occurred, followed by cardiogenic shock. It is not clear whether these cardiovascular effects were due to a direct action of endosulfan on the cardiovascular system or a result of a more general toxic insult (e.g., convulsions). It is also unclear whether other ingredients in the Thionax® may have contributed to the effects observed. A similar picture was described in another lethal case of acute intoxication with endosulfan (Lo et al. 1995). Severe cardiovascular effects developed in a

woman who ingested an unknown amount of endosulfan mistakenly mixed into food (Blanco-Coronado et al. 1992). On admission to the hospital, she had transient hypotension (60/30 mm Hg). Over the next few days her hemodynamic parameters remained abnormal, and she died 8 days after admission following acute renal failure, disseminated intravascular congestion, thrombi in the pulmonary arteries and aorta, and cardiogenic shock. A man who ingested approximately 260 mg/kg endosulfan experienced a drop in arterial blood pressure on the day of exposure, and focal cardiac inflammation and "slight" heart congestion on autopsy 4 days after exposure (Boereboom et al. 1998). No other information was located regarding the cardiovascular effects of ingested endosulfan in humans.

Male rats given a single oral dose of 200 mg/kg of endosulfan had myocardial hemorrhages (Terziev et al. 1974). It is not clear whether this effect was due to a direct effect of endosulfan on the heart or secondary to other toxicity such as damage occurring in response to effects of endosulfan on neural control of the heart.

In general, longer-term exposure of animals to sublethal concentrations of endosulfan has not resulted in gross or microscopic evidence of cardiovascular toxicity (FMC 1959a, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989c). However, three rat studies indicated possible toxic effects. Male rats that consumed 0.75 mg/kg/day for at least 84 days had an increase in relative heart weight (Hoechst 1984a). Also, male rats that consumed 2.9 mg/kg/day for 2 years had an increased incidence of aneurysms in blood vessels (Hoechst 1989a). Female rats were not similarly affected at doses up to 3.8 mg/kg/day for 2 years (Hoechst 1989a). In light of the large number of negative studies that used similar doses of endosulfan, the biological significance of the isolated observations of blood vessel aneurysms is unknown. An additional chronic study in rats, that used larger doses (20 and 48 mg/kg/day), reported calcification of the heart and the aorta and mesenteric arteries in male rats (NCI 1978). The calcification was thought to be caused by parathyroid hyperplasia, which in turn was secondary to kidney disease.

Gastrointestinal Effects. Nausea, gagging, vomiting, and diarrhea were part of the clinical syndrome exhibited by persons who consumed high doses (lethal in some cases) of endosulfan either intentionally or accidentally (Blanco-Coronado et al. 1992; Pradhan et al. 1997; Terziev et al. 1974). However, it is unclear whether these effects were the result of gastrointestinal irritation or were mediated by effects of endosulfan on central nervous system control of gastrointestinal function. Mucosal inflammation of the stomach and the proximal small intestinal were postmortem observations in a man who purposely ingested an unknown amount of endosulfan (Lo et al. 1995). In contrast, a man who

ingested endosulfan once at approximately 260 mg/kg did not show any apparent stomach or intestinal lesions at autopsy 4 days later (Boereboom et al. 1998).

Female rats that received a single gavage dose of 63 mg of β-endosulfan/kg, a dose which was lethal, had blood in the small intestines and mucus in the stomach (Hoechst 1988a). Studies in dogs indicate that acute exposure to relatively high doses of endosulfan may cause stomach irritation and vomiting. Dogs that consumed 2.5 mg/kg/day for 3 days vomited (FMC 1959a), and dogs given a single oral dose of 30 mg/kg exhibited vomiting and stomach irritation (FMC 1958). Following a single oral dose of 50 mg/kg, dogs had congestion in the stomach and small intestine (Hoechst 1970). Similarly, rats given single unspecified doses of endosulfan in an LD_{50} determination showed irritant gastroenteritis (Boyd et al. 1970). Rabbits treated with a single dose of 15.1 mg technical endosulfan/kg had watery diarrhea for 3–4 days after dosing, but eventually recovered (Ceron et al. 1995); this dose level was lethal to some of the treated rabbits.

Longer-term exposure of animals to sublethal doses of endosulfan has generally not resulted in observable signs of gastrointestinal toxicity. Routine gross and histopathological examination of the gastrointestinal tract revealed no adverse effects in rats, mice, or dogs in such studies (FMC 1959a, 1959b, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989a, 1989c). However, convulsive spasms of the abdominal and jaw muscles without vomiting were observed in male and female dogs that consumed 2.0 mg/kg/day and 1.8 mg/kg/day, respectively, for 1 year (Hoechst 1989c). No adverse gross or histopathological findings were noted following examination of the gastrointestinal tracts of these animals, indicating that the spasms may have been a neurological effect rather than the result of gastrointestinal irritation.

Hematological Effects. Leukocytosis and decreased platelet counts were reported in a group of subjects shortly after they ingested an unknown amount of endosulfan (Blanco-Coronado et al. 1992). One subject from that study, who eventually died, had prolonged partial thromboplastin time and prothrombin time with thrombocytopenia, and decreased fibrinogen two days after being admitted to the hospital. Elevated white cell count was also observed in an additional case of fatal acute poisoning with endosulfan (Lo et al. 1995). Significantly elevated hemoglobin (61.2 g/100 mL compared to a reference range of 13–18 g/100 mL) and slightly elevated white cell count (12,600/mm^3 compared to a reference range of 5000–10,000/mm^3), but normal hematocrit, were seen in a male patient at approximately 40 minutes after ingesting 260 mg endosulfan/kg; the man subsequently died (Boereboom et al. 1998).

No further information was located regarding hematological effects in humans after oral exposure to endosulfan.

Treatment-related effects on red blood cells have been noted following high-dose acute-duration oral exposure of animals to endosulfan. For example, decreased erythrocyte Na^+-K^+ ATPase activity was observed in female rats treated daily with 12.5 mg technical endosulfan/kg for 4 days (Kiran and Varma 1988). Female rats treated with a single gavage dose of 22 mg endosulfan/kg had decreased hemoglobin at sacrifice 24 hours after dosing; a group treated with a 33 mg/kg dose showed decreases in red blood cells, hemoglobin, and packed cell volume (Siddiqui et al. 1987b); however, these dose levels may have been close to lethal doses. Rabbits administered a single gavage dose of 15.1 mg endosulfan/kg also showed a decrease in red blood cells, hemoglobin, and packed cell volume; this dose was lethal to 5 out of 7 rabbits (Ceron et al. 1995).

Mixed results have been obtained in studies examining longer-term exposures to endosulfan. Adverse hematological effects were observed in a well conducted study in which rats were administered endosulfan in the diet for 13 weeks (Hoechst 1985a). At 6 weeks, effects observed in male rats that consumed 1.9 mg/kg/day included decreased hemoglobin, red blood cell count, and mean corpuscular hemoglobin concentration, and increased mean corpuscular volume. Decreased mean corpuscular hemoglobin concentration was observed in female rats at a similar dose. At higher doses in this study, the magnitude of the effects increased, and effects comparable to those observed in males were observed in females. At 13 weeks, males exhibited decreased hemoglobin concentration at 3.8 mg/kg/day and above, whereas decreased hemoglobin was seen in females at 0.8 mg/kg/day. Following a 4-week withdrawal period, spleen weights were significantly increased in males at $1.9 mg/kg/day. However, hematological determinations performed in other intermediate- and chronic-duration studies in rats using doses comparable to those noted above do not support the ability of endosulfan to cause anemia (Das and Garg 1981; Dikshith et al. 1984; FMC 1959b; Hack et al. 1995; Hoechst 1989a). In fact, increased red blood cell count was observed in male rats treated with 5 mg of technical endosulfan/kg/day for 30 days (Dikshith et al. 1984). Also, no effects on hematological parameters or on routine gross and histopathological examination of bone marrow and the spleen were observed in mice or dogs during intermediate- and chronic-duration studies (FMC 1959a, 1967; Hoechst 1984b, 1988b, 1989c).

The adverse effects on the blood observed in the study by Hoechst (1985a) cannot be totally discounted as spurious. A possible explanation for the discrepancy between the findings in the Hoechst study (1985a) and the other studies noted above may be provided by the results of the study by Das and Garg

(1981). These authors found decreased red blood cells in rats reared on a low-protein diet (3.5% protein) that contained endosulfan at levels calculated to be equivalent to doses of 0.025 and 5 mg/kg/day of endosulfan for 9–18 weeks. However, no effect was observed at these doses in rats given normal protein diets prior to exposure, indicating that protein deficiency enhances the anemia-inducing capacity of endosulfan. Thus, some subtle stressor may have affected the response of rats in the study by Hoechst (1985a) such that they responded similarly to rats that consumed a protein-deficient diet.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans following oral exposure to endosulfan. Only limited information was obtained regarding effects of endosulfan on muscle and/or bone in animals. Routine gross and microscopic examination of samples of bone and/or muscle obtained from animals in intermediate-duration (Hoechst 1984b, 1989c) and chronic-duration (FMC 1967; Hoechst 1988b, 1989a, 1989c) studies revealed no adverse effects of endosulfan on these tissues.

Hepatic Effects. Elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were reported in a woman 2 days after being admitted to the hospital because of ingestion of endosulfan-contaminated food (Blanco-Coronado et al. 1992). The patient died 8 days after admission, following acute renal failure, disseminated intravascular coagulation, thrombi in the pulmonary arteries and aorta, and cardiogenic shock. Postmortem examination revealed dilation and congestion of hepatic sinusoids. Centrilobular congestion and slight prominence of the bile canaliculi were among postmortem observations in an additional fatal case of acute poisoning with endosulfan (Lo et al. 1995). A man who ingested approximately 260 mg/endosulfan/kg showed liver congestion on autopsy 4 days after exposure (Boereboom et al. 1998). No further information was located regarding hepatic effects in humans after oral exposure to endosulfan.

Studies in experimental animals indicate that both toxic effects and adaptive effects may be seen in the liver following oral exposure to endosulfan.

Autopsy of dogs that ingested single lethal doses of endosulfan (10 mg/kg, FMC 1958; 50 mg/kg, Hoechst 1970) revealed liver congestion. Similarly, autopsied rats that received unspecified doses of endosulfan in an LD_{50} study were reported to have liver congestion (Boyd et al. 1970). Rats receiving a single oral dose of 33 mg/kg of endosulfan had increased serum glutamate-pyruvate transferase activity, indicating hepatic damage (Siddiqui et al. 1987b). Female rats treated daily by gavage with 12.5 mg technical endosulfan/kg for 4 days showed decreased activity of liver aldolase (Kiran and Varma 1988).

Rabbits that were administered a single gavage dose of 15.1 mg of technical endosulfan/kg, a dose that caused severe general toxicity, had significantly increased serum alkaline phosphatase (AP), ALT, and AST activities (suggesting liver damage) in the days following treatment (Ceron et al. 1995); no histopathology was conducted in this study. These observations are consistent with findings in humans acutely exposed to high doses of endosulfan.

Adaptive effects (including increased microsomal enzyme activity, increased liver weight, increased smooth endoplasmic reticulum, and decreased pentobarbital-induced sleeping time) in the absence of any signs of toxicity have also been observed in female rats in acute-duration studies at doses as low as 2.5 mg/kg/day for 7 days (Gupta and Gupta 1977a) or 14 days (FMC 1980a) and in male rats at doses as low as 5 mg/kg/day for 2 days (Misra et al. 1980) or 10 mg/kg/day for 14 days (Den Tonkelaar and Van Esch 1974).

Increased liver weight has also been observed in several intermediate-duration studies. For example, increased liver weight has been observed in female rats exposed to 2.5 mg/kg/day for 15 days (Gupta and Gupta 1977a) and in maternal animals exposed to 0.75 mg/kg/day endosulfan for approximately 84 days (Hoechst 1984a). In male rats, increased liver weight was observed at doses as low as 5 mg/kg/day after 15 days (Gupta and Chandra 1977) or 30 days (Dikshith et al. 1984) and at doses as low as 3.75 mg/kg/day for approximately 84 days (Hoechst 1984a). In a more recent study, doses of 3 mg technical endosulfan/kg/day in the food for 30 days significantly increased serum and liver AP, AST, and ALT activities in female rats, but not in males (Paul et al. 1995); these effects were seen in the males at a dose level of 6 mg/kg/day. The seemingly greater toxicity in the females was attributed to differences in metabolism between males and females. Evidence of microsomal enzyme induction (decreased pentobarbital-induced sleeping time) was also observed in female rats at 2.5 mg/kg/day for 30 days (Gupta and Gupta 1977a). In general, increases in liver weight have not been accompanied by adverse histopathological changes (Dikshith et al. 1984; Hoechst 1984a); however, exposure of male rats to 5 mg/kg/day for 15 days was reported to result in moderate dilation of the sinusoids, areas of focal necrosis, Kupffer cell hyperplasia, and bile duct proliferation with more severe necrosis, inflammation, and dilation at 10 mg/kg/day (Gupta and Chandra 1977). Also, increased serum AP was observed in male and female rats in the study by Dikshith et al. (1984), suggesting that both adaptive and toxic effects may be observed in some intermediate-duration studies.

In studies of somewhat longer duration, effects on liver weight were not observed or were observed only at high doses. Exposure of female rats to doses as high as 5 mg/kg/day for up to 18 weeks and male rats

to doses as high as 3.85 for 13 weeks (Hoechst 1985a) had no effect on liver weight (Das and Garg 1981; Hoechst 1985a). Increases in liver weight were observed after 13 weeks only at doses of 23.41 mg/kg/day in males and 27.17 mg/kg/day in females (Hoechst 1985a). In this study, granular brown pigment was observed in livers of males at 23.41 mg/kg/day, and centrilobular enlargement was observed in livers of females at 27.17 mg/kg/day; however, these changes were no longer apparent following a 4-week "withdrawal" period during which the animals were no longer exposed to endosulfan. In mice, an increase in liver weight was observed in females at 4.6 mg/kg/day but not in males at doses as high as 3.7 mg/kg/day for 42 days (Hoechst 1985b). No effect on mouse liver weight was observed at doses as high as 7.3 mg/kg/day (males) and 7.52 mg/kg/day (females) for 13 weeks (Hoechst 1984b). In both mouse studies, no adverse histopathological findings were observed during routine microscopic examination of the livers. Similarly, no adverse histopathological findings were observed during routine microscopic examination of the livers of dogs exposed to TWA doses of 2.9 mg/kg/day (males) and 2.6 mg/kg/day (females) for 146 days (Hoechst 1989c), but serum alkaline phosphatase was elevated in females treated with the 2.6 mg/kg/day dose.

Chronic-duration studies have generally not shown adaptive or adverse effects on the liver. Routine gross and microscopic pathology has not revealed adverse hepatic effects in mice exposed to 2.51 mg/kg/day (males) or 2.86 mg/kg/day (females) for 2 years (Hack et al. 1995; Hoechst 1988b), in rats exposed to #5 mg/kg/day (females) or 2.9 mg/kg/day (males) for 2 years (Hack et al. 1995; FMC 1959a; Hoechst 1989a), or in dogs exposed to 1 mg/kg/day for 2 years (FMC 1967). Serum alkaline phosphatase was, however, elevated in dogs exposed to 0.67 mg/kg/day (males) or 0.6 mg/kg/day (females) for 1 year, suggesting adverse effects on the liver; however, no effects on liver weight, liver function, or microscopic pathology were observed (Hoechst 1989c). A chronic-duration oral MRL of 0.002 mg/kg/day was derived based on the NOAEL level of 0.18 mg/kg/day determined in this study. An increase in the incidence of hydropic hepatic cells in the liver of male rats exposed to 5 mg/kg/day for 2 years (FMC 1959b) was also observed, indicating that hepatic toxicity may be observed in chronic studies when sufficiently high doses are administered.

Renal Effects. Hemorrhage of the medullary layer of the kidneys was reported in three persons who died following ingestion of endosulfan (Terziev et al. 1974). Acute renal failure was a major contributor to the deaths of two individuals who ingested unknown amounts of endosulfan (Blanco-Coronado et al. 1992; Lo et al. 1995). In both cases, postmortem examination revealed extensive tubular necrosis. In contrast, no kidney lesions were found in a man who died 4 days after ingesting approximately 260 mg endosulfan/kg (Boereboom et al. 1998).

Ingestion of acutely lethal doses of endosulfan has also been associated with renal hemorrhage and congestion in studies in rats and dogs (FMC 1958, 1980a; Hoechst 1970; Terziev et al. 1974). Hemorrhagic areas on the kidney were observed at doses as low as 20 mg/kg/day for 14 days in a range-finding study using small numbers of pregnant rats (FMC 1980a). Congestion and hypertrophy were observed following a single dose as low as 10 mg/kg in a study using a limited number of dogs (FMC 1958).

In intermediate-duration studies in rats, congestion and focal degeneration in the epithelial lining of kidney tubules were observed in males treated with doses of 10 mg/kg/day for 15 days (Gupta and Chandra 1977). Also, yellow protein aggregates in the lumen and eosinophilic droplets in the cells of some proximal convoluted tubules were observed in rats following consumption of a diet that provided 3.9 mg/kg/day of technical endosulfan for 13 weeks (Hoechst 1985a). At 23.4 mg/kg/day males exhibited proteinuria (Hoechst 1985a). At lower doses, however, effects in rats have been limited to increases in kidney weight and changes in cellular pigmentation (Dikshith et al. 1984; FMC 1965; Hoechst 1984a, 1985a). Increases in relative kidney weight have been observed in rats at doses as low as 3.75 mg/kg/day for 84 days (Hoechst 1984a). Increases in yellow discoloration of the cytoplasm of cells of the proximal convoluted tubules have been observed following consumption of doses as low as 0.64 mg/kg/day for 13 weeks by male rats (Hoechst 1985a). Granular clumped pigment was also observed in cells of the straight portions and occasionally in the proximal convoluted tubules in male rats in this study at doses as low as 3.85 mg/kg/day at the end of 13 weeks of exposure and at doses as low as 1.92 mg/kg/day at the end of the 4-week withdrawal period. In mice, consumption of 7.3 mg/kg/day (males) or 7.52 mg/kg/day (females) for 13 weeks resulted in no gross or microscopically evident adverse effects (Hoechst 1984b). Similarly, in dogs given TWA doses of 2.9 mg/kg/day (males) or 2.6 mg/kg/day (females) for 146 days, routine gross and histopathological examination of the kidneys and urinary bladder revealed no adverse effects (Hoechst 1989c).

The toxicological relevance of the yellow discoloration of the cytoplasm of the cells of the proximal convoluted tubules and the increase in relative kidney weight that was observed in the study by Hoechst (1985a) was investigated in a subsequent study (Hoechst 1987) because toxicokinetic studies indicated that endosulfan accumulated in the kidneys of animals following intermediate-duration exposure (Ansari et al. 1984; Dorough et al. 1978; Nath and Dikshith 1979). Thus, the yellow discoloration and increase in kidney weight may have been merely a reflection of endosulfan storage within the cells of the proximal convoluted tubules rather than a toxic effect. In the latter study, light and electron microscopy of the kidneys of rats that consumed either 34 or 68 mg/kg/day for 4 weeks showed pigment deposits and an

increase in the number and size of lysosomes in the cells of the proximal convoluted tubules (Hoechst 1987). Other cell compartments were not affected, and the lysosomes were not reported to contain membrane fragments, indicating the absence of significant renal cell damage. A simultaneous determination of endosulfan residues in the kidney showed that α-endosulfan levels in the kidney were quite high relative to levels in the liver and blood. Relative kidney weights were also increased when compared to the controls. When given a 30-day recovery period, the kidney weight, yellow pigmentation, size and number of lysosomes, and the levels of α-endosulfan in the kidneys decreased considerably. The authors interpreted these results as showing that, at the doses and the exposure duration tested, endosulfan accumulates in the kidney without causing detectable toxicity. When given an endosulfan-free recovery period, the storage dissipated (i.e., the yellow discoloration and detectable levels of endosulfan were no longer observed in many of the rats). The increased kidney weights were proposed to reflect the increased storage and/or metabolism of the endosulfan. Therefore, based on the absence of evidence of autophagy of damaged organelles, the yellow discoloration and increases in the relative kidney weight were considered to be adaptive effects similar to the increases in liver weight and microsomal enzyme activity produced by many xenobiotic substances. However, no assessment of renal function was performed in this study. Therefore, it remains unclear whether the yellow discoloration occurs in the absence of renal toxicity.

Minor changes of questionable biological significance observed in rats administered 5 mg/kg/day of endosulfan in a low-protein diet for 9 weeks include a decrease in capsular space and an increase in perirenal adipose tissue (Das and Garg 1981).

Chronic ingestion of endosulfan by rats has been reported to result in nephrotoxicity. Consumption of technical-grade endosulfan by rats for 78 weeks (followed by a 33-week observation period) at TWA doses of 20 mg/kg/day (males) and 11.1 mg/kg/day (females) resulted in toxic nephropathy characterized by degenerative changes in the proximal convoluted tubules at the junction of the cortex and medulla (NCI 1978). Cloudy swelling, fatty degeneration, and necrosis of the tubular epithelium were also evident. Reuber (1981) re-analyzed the histological sections from the NCI study, and found that chronic renal fibrosis was evident in 100% of exposed male rats, and that there was a significantly increased incidence of female rats with acute necrosis of the tubules. Similar results were obtained at lower doses in male rats in studies by FMC (1959b) and Hoechst (1989a). At doses of 5 mg/kg/day for 2 years an increase in kidney weight, renal tubule dilation, albuminous casts, focal interstitial nephritis, and degeneration of renal tubule epithelium were observed in male rats (FMC 1959b). Similarly, at doses of approximately 3 mg/kg/day for 2 years, progressive glomerulonephrosis was observed in male rats (Hack

et al. 1995; Hoechst 1989a). No evidence of renal toxicity was observed in female rats in these studies following consumption of doses of 5 mg/kg/day (FMC 1959b) or 3.8 mg/kg/day (Hack et al. 1995; Hoechst 1989a) for 2 years other than enlarged kidneys. These results indicate that male rats are more susceptible to the renal toxicity of endosulfan than female rats.

In contrast to the effects seen in rats following chronic-duration exposure, mice and dogs have not shown any evidence of nephrotoxicity at the doses that have been tested. Ingestion by mice of doses of endosulfan of 2.51 mg/kg/day (males) and 2.86 mg/kg/day (females) for up to 2 years resulted in no grossly or microscopically evident adverse effects on the kidneys or urinary bladder (Hack et al. 1995; Hoechst 1988b; NCI 1978). Similarly, ingestion by dogs of doses as high as 2 mg/kg/day (males) and 1.8 mg/kg/day (females) for 1 year (FMC 1959a; Hoechst 1989c) or 1 mg/kg/day (males and females) for 2 years (FMC 1967) resulted in no evidence of nephrotoxicity. Thus, rats appear to be more sensitive to the nephrotoxic effect of endosulfan.

Endocrine Effects. No studies were located regarding endocrine effects in humans following oral exposure to endosulfan.

Administration of a single oral dose of 5 mg of endosulfan/kg to rats resulted in degranulation of the β-cells of the islets of Langerhans of the pancreas (Barooah et al. 1980). This effect, however, was not observed after the same dose was administered daily for five days. Both administration protocols caused dilation of the blood vessels of the islets of Langerhans. Administration of 5 mg technical endosulfan/kg/day for 7 days to rats did not significantly alter the weight of the adrenals (Gupta and Gupta 1977a).

Routine gross and/or microscopic examination of the adrenals, pituitary, thyroid, or parathyroid did not reveal any adverse effects following intermediate exposure of rats, mice, or dogs to doses ranging from 2.5 to 10 mg/kg/day (FMC 1965; Gupta and Chandra, 1977; Hoechst 1984b, 1988b, 1989c). Similar lack of effects were reported in rats administered up to 5 mg endosulfan/kg/day for up to 2 years (FMC 1959b; Hoechst 1989a), dogs treated with up to 1 mg/kg/day for 2 years (FMC 1967) or mice administered 2.5 mg/kg/day for 2 years (Hoechst 1988b). Parathyroid hyperplasia and mineralization (calcium deposits) in several tissues were observed in male rats treated for 74–82 weeks, with estimated doses of 20 mg technical endosulfan/kg/day (NCI 1978). Both of these lesions were secondary to chronic renal failure (NCI 1978). Reuber (1981) re-evaluated the histological sections from the NCI study, and

indicated that the incidence of rats with parathyroid hyperplasia was significantly increased at both treatment levels among males, but not among females.

Endosulfan administered by gavage at 1.5 mg/kg/day for 30 days to ovariectomized rats did not influence the relative weights or histology of the uterus, cervix, or vagina compared to ovariectomized control rats that did not receive endosulfan (Raizada et al. 1991). Rats in a positive control group received intraperitoneal injections of estradiol and showed increased relative organ weights and normal development of female reproductive tissues compared to the untreated ovariectomized control rats. Organ weights and tissue development in rats administered both endosulfan and estradiol were not significantly different from those seen in rats that received estradiol alone. The study results indicate that endosulfan was neither estrogenic nor anti-estrogenic under the conditions of this assay.

Significantly increased serum testosterone and decreased testicular testosterone were reported in male rats after a 7-day exposure to endosulfan using oral doses in the range of 7.5–10 mg/kg/day, but not at #5 mg/kg/day (Singh and Pandey 1989). However, results after a 15-day exposure were highly variable and frequently not dose-related, making interpretation of the significance of the study's results difficult. A subsequent study (Singh and Pandey 1990) indicated a dose-related decrease in testicular testosterone, plasma testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH) in groups of male Wistar rats orally administered endosulfan at 0, 7.5, or 10 mg/kg/day for 15 or 30 days. In addition, activities of steriodogenic enzymes and testicular cytochrome P450-dependent monooxygenases were depressed after the 30-day exposure at $7.5 mg/kg/day. All of the effects from 30 days of exposure were reversible during a 7-day recovery period, except for decreased testicular testosterone, which remained depressed; no recovery period was utilized for the 15-day exposures.

Dermal Effects. No studies were located regarding dermal effects in humans following oral exposure to endosulfan.

Only limited information was obtained regarding the effects of endosulfan on the skin in animals. Routine gross and microscopic examination of samples of skin obtained from dogs treated with 2.6 mg endosulfan/kg/day in the diet for 147 days revealed no adverse effects (Hoechst 1989c). Female, but no male, rats treated for 13 weeks with 4.6 mg endosulfan/kg/day in the diet exhibited hair loss in the dorsal scapular and cervical regions (Hoechst 1985a). Chronic treatment of rats, mice, or dogs with doses of approximately 2 mg endosulfan/kg/day caused no significant alterations in the skin (Hoechst 1988b, 1989a, 1989c).

Ocular Effects. No studies were located regarding ocular effects in humans following oral exposure to endosulfan.

Only limited information was obtained regarding the effects of endosulfan on the eyes in animals. Routine gross and microscopic examination of samples of eyes obtained from rats, mice, and dogs in intermediate-duration (FMC 1965; Hoechst 1984b, 1985b, 1989c) and chronic-duration (Hoechst 1988b, 1989a, 1989c) studies revealed no adverse effects of endosulfan on these tissues. Also, ophthalmoscopy of the eyes revealed no treatment-related effects in rats that consumed doses of up to 23.41 mg/kg/day (males) and 27.17 mg/kg/day (females) for 13 weeks (Hoechst 1985a) or 2.9 mg/kg/day (males) and 3.8 mg/kg/day (females) for 2 years (Hack et al. 1995; Hoechst 1989a); in mice that consumed 3.7 mg/kg/day (males) and 4.6 mg/kg/day (females) for 42 days (Hoechst 1985b); or in dogs that consumed TWA doses of 2.9 mg/kg/day (males) and 2.6 mg/kg/day (females) for 146 days or 2 mg/kg/day (males) and 1.8 mg/kg/day (females) for 1 year (Hoechst 1989c).

Body Weight Effects. No studies were located regarding body weight effects in humans following oral exposure to endosulfan.

Body weight was not significantly affected in rats treated with up to 6 mg endosulfan/kg/day for 7–8 days (Gupta and Gupta 1977a; Lakshmana and Raju 1994) or in mice treated with up to 15 mg technical endosulfan/kg in the food for 7 days (Wilson and LeBlanc 1998). However, rabbits treated once with 15.1 mg technical endosulfan/kg by gavage and followed for 35 days exhibited a 12% reduction in body weight (Ceron et al. 1995); the dose level was lethal in the rabbit study. No significant effects on body weight were obtained in intermediate-duration studies in which rats were administered 5 mg endosulfan/kg/day by gavage for 15 days (Gupta and Gupta 1977a), 1.5 mg endosulfan/kg/day by gavage for 30 days (Dikshith et al. 1984), or 5 mg endosulfan/kg/day in the diet for 30 days (Paul et al. 1995) or 9–18 weeks (Das and Garg 1981) or 10 mg endosulfan/kg by gavage in oil 5 days/week for 90 days (Sinha et al. 1997). Contradicting the results of Sinha et al. (1997), in a study by Gupta and Chandra (1977), rats treated with 10 mg endosulfan/kg/day by gavage in oil for 15 days gained 30% less weight than control; this dose also caused lethality. Also, decreased body weight gain was reported in dams treated with doses of 3.8 mg/kg/day for 84 days (Hoechst 1984a). A dose of 2 mg technical endosulfan/kg/day by gavage in water for 90 days was also reported to cause significant reduction in weight gain in rats (Paul et al. 1994); in this case, food intake was also suppressed. Body weight gain was significantly reduced in male, but not female mice treated in the diet with 2.5 mg endosulfan/kg/day for 24 months (Hack et al. 1995; Hoechst 1988b); the no-effect-level dose was 0.8 mg/kg/day. Both male

and female rats treated with 0.6–0.7 mg endosulfan/kg/day in the diet for 24 months also exhibited reduction in body weight gain in the range of 11–15% (Hack et al. 1995); dose levels between 3 and 3.5 mg/kg/day caused body weight gain reductions in the range of 21–29%. The no-effect-level dose was approximately 0.3 mg/kg/day. An additional chronic study also reported a significant decrease in weight gain in male rats fed a diet that provided approximately 20 mg of technical endosulfan/kg/day (NCI 1978). In this case, the treated animals were approximately 23% lighter than matched controls after 80 weeks on the experimental diet.

Metabolic Effects. Severe metabolic acidosis with high anion gap and hyperglycemia was reported in humans after acute poisoning with endosulfan (Blanco-Coronado et al. 1992; Lo et al. 1995). In five of the six cases reported by Blanco-Coronado et al. (1992), the metabolic acidosis was corrected with gastric lavage with activated charcoal and intravenous sodium bicarbonate and diazepam. No further information regarding metabolic effects in humans after exposure to endosulfan was located.

Studies in animals indicate that this chemical may affect glucose metabolism and ion permeability of cells. Increased blood glucose and/or decreased hepatic glycogen levels have been observed following acute- and intermediate-duration oral exposure to endosulfan (Chatterjee et al. 1986; Garg et al. 1980; Kiran and Varma 1988). It should be noted that this has been observed in animals exhibiting frank neurotoxicity. Interestingly, the hyperglycemia and decreased hepatic glycogen levels reported by Kiran and Varma (1988) were much more marked in older rats than in younger animals; and older animal, but not younger ones showed frank neurotoxic effects. Decreased serum glucose levels and degranulation of the β-cells of the islets of Langerhans of the pancreas (indicating release of insulin) were observed following a single dose, but not multiple doses, of endosulfan (Barooah et al. 1980). The observation of increases in serum glucose in some studies but decreases in others may be due to the differences in the doses of endosulfan used in these studies. The doses at which increases in serum glucose were observed (12.5–70 mg/kg/day) were higher than those at which a decrease in serum glucose was observed (5 mg/kg/day).

Decreased serum calcium has also been observed following a 7-week oral exposure to 5 mg/kg/day of endosulfan (Garg et al. 1980).

Other Systemic Effects. In a group of 7 rabbits treated with a single dose of 15.1 mg technical endosulfan/kg by gavage in oil, 2 that recovered from the severe initial neurotoxic effects decreased their food intake by 82% relative to controls during the following weeks after treatment (Ceron et al. 1995).

The remaining 5 rabbits died within hours of dosing. Rats treated for 90 days with daily doses of 2 mg technical endosulfan/kg by gavage in water also reduced their food intake throughout the study (Paul et al. 1994). On the average, the treated rats ate 22% less food than the controls. Although the treated rats did not exhibit severe neurotoxic effects, their spontaneous motor activity was increased relative to controls. Neither food nor water consumption was significantly altered in mice or rats administered technical endosulfan in the diet for 24 months (Hack et al. 1995). In spite of this finding, both mice and rats gained significantly less weight during the study than their matched controls.

2.2.2.3 Immunological and Lymphoreticular Effects

No lesions of the spleen were evident on autopsy of a man who ingested a dose of approximately 260 mg endosulfan/kg (Boereboom et al. 1998).

Studies in male rats indicate that both humoral and cellular immune responses are depressed by endosulfan at doses that do not induce any other overt signs of toxicity. In a series of experiments, Banerjee and Hussain (1986, 1987) administered endosulfan in the diet of male rats at concentrations ranging from 5 to 50 ppm (equivalent to 0.45–4.5 mg/kg/day) for 6–22 weeks. The animals were immunized with a subcutaneous injection of tetanus toxin with an equal volume of Freund's complete adjuvant approximately 20 days prior to sacrifice. The animals did not exhibit any overt signs of toxicity, and no changes in body weight or mortality were noted. Serum antibody titer (to tetanus toxin), serum immunoglobulin levels (IgM and IgG), and serum globulin fractions (α-, β-, and γ-globulin) were studied to evaluate humoral immune responses. Serum antibody titer to tetanus toxin, IgG, IgM, and γ-globulin levels were significantly decreased in rats exposed to 4.5 mg/kg/day of endosulfan for 6 weeks and in rats exposed to 0.9 mg/kg/day of endosulfan for longer periods. The effects of endosulfan on cell-mediated immune competence were evaluated with macrophage migration inhibition (MMI) and leukocyte migration inhibition (LMI) tests. The results of both tests indicated that the cell-mediated immune response was significantly depressed in a dose-related manner in animals administered 1.8, 2.7, and 4.5 mg/kg/day. Spleen and thymus weights were not affected by endosulfan treatment in animals treated for 6 weeks, but a significant decrease in spleen weight was observed at 22 weeks in the 1.8 mg/kg/day dose group. These rats also had a significantly increased albumin-to-globulin ratio at week 22. The authors concluded that these results indicate that endosulfan can suppress both humoral and cell-mediated immune responses in rats exposed to levels of endosulfan that induce no other signs of toxicity. This was an apparently well conducted study that measured sensitive indicators of both humoral and immune function using doses of endosulfan that have not been previously shown to cause toxicity. Male

mice exposed to 7.3 mg/kg/day for 13 weeks had significantly decreased spleen weights and decreased neutrophil counts (Hoechst 1984b), indicating that immune activity in mice may also be affected. An intermediate-duration oral MRL of 0.005 mg/kg/day was derived based on the NOAEL of 0.45 mg/kg/day for immunotoxicity identified in the Banerjee and Hussain (1986) study. In support of these positive findings, Khurana et al. (1998) observed decreased macrophage functionality, in the absence of any other apparent toxicological effects, in 1-day-old broiler chicks fed 30 ppm endosulfan in the diet for 4 or 8 weeks.

Other studies have examined the effects of endosulfan on immune function in rats and have not observed effects at higher doses; however, these other studies have examined the effects of endosulfan administration for shorter durations and did not evaluate many of the same end points that showed positive effects in the studies by Banerjee and Hussain (1986, 1987). For example, doses as high as 4.5 mg/kg/day given 2 days before and 10 days after infection with *Trichinella spiralis* larvae resulted in no effect on the number of worms found in the body at sacrifice, no effect on the thymus or spleen weights, and no effect on the percent lymphocytes or white blood cell count (Hoechst 1988c). Also, there were no or marginal effects on the weight and histopathology of the thymus, spleen, or mesenteric and popliteal lymph nodes, or on leukocyte or monocyte counts. Serum IgM and IgG were not affected by 3 weeks of exposure to 5 mg/kg/day (Vos et al. 1982).

Also, chronic-duration studies have not generally shown adverse effects on organs of the immune system. Routine gross and histopathologic examination of the lymph nodes and thymus of rats, mice, and dogs exposed to endosulfan for 2 years at doses of up to 2.9 mg/kg/day (Hoechst 1989a), 2.51 mg/kg/day (Hoechst 1988b), and 1 mg/kg/day (FMC 1967), respectively, revealed no adverse effects. However, these studies did not assess immune function directly.

These results demonstrate that immunotoxicity may be a sensitive end point of endosulfan-induced toxicity following exposure to low doses for sufficient durations. The highest NOAEL value and all reliable LOAEL values for immunological effects in each species in each duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

The most prominent signs of acute overexposure to endosulfan in both humans and animals are hyperactivity, tremors, decreased respiration, dyspnea, salivation, and tonic-clonic convulsions. Five

cases of acute lethal poisoning in humans resulting from accidental or intentional ingestion of Thiodan® were reported in an early study by Terziev et al. (1974). The ingested doses were not specified. Initial clinical signs observed in all cases included nervous system effects such as agitation, writhing, and loss of consciousness. Autopsies performed in three of the cases revealed brain edema. Central nervous system stimulation also characterized the clinical syndrome displayed by a 20-year-old man who attempted suicide by ingesting 200 mL of a 30% endosulfan formulation (Thionax®) (Shemesh et al. 1988). Although the patient's stomach contents were aspirated and he was given activated charcoal to reduce absorption, in the first 2 weeks following ingestion, the patient displayed recurrent convulsions. This stage was followed by a slow recovery phase, in which psychomotor function slowly returned. One year after his attempted suicide, his mental activity (presumably psychomotor activity) was still severely impaired, and he required medication to control his seizures. This case report demonstrates that long-term brain damage can occur following acute overexposure to endosulfan in humans. The brain damage may have been a result of a direct action of endosulfan on the brain tissue or the hypoxia that accompanied the recurring seizures and respiratory insufficiency seen within the first 2 weeks of ingestion. It is also unclear whether the effects observed may have been due, in part, to other ingredients in the Thionax®.

Similarly, convulsive seizures and a sustained epileptic state persisted after stomach contents were pumped and activated charcoal and anticonvulsive medication were administered in a 43-year-old man who ingested approximately 260 mg/kg endosulfan (Boereboom et al. 1998). At 4 days after exposure, the man was pronounced brain dead, and autopsy revealed cerebral hernia from massive cerebral edema. Eight additional accidental and/or intentional cases of acute poisoning with endosulfan resulting in adverse neurological effects have been reported in more recent studies, six by Blanco-Coronado et al. (1992), one by Lo et al. (1995), and one by Pradhan et al. (1997); two out of the eight resulted in death. Tonic-clonic convulsions were seen in the Blanco-Coronado et al. (1992) cases, whereas Lo et al. (1995) reported the development of muscle fasciculations and episodes of convulsions in their case. In the case reported by Pradhan et al. (1997), the patient had consumed about 75 mL of liquid endosulfan (35% w/v). In this case, in addition to tonic-clonic seizures and myoclonic jerks, the patient developed psychosis, cortical blindness and limb rigidity. Magnetic resonance imaging showed reversible lesions of the basal ganglia and occipital cortex. The amount of endosulfan ingested in the Blanco-Coronado et al. (1992) and Lo et al. (1995) reports was unknown.

Central nervous system stimulation is the hallmark of acute overexposure to endosulfan in experimental animals. The spectrum of effects includes hyperexcitability, tremors, decreased respiration, tonic-clonic convulsions, and ultimately, death (Boyd and Dobos 1969; Boyd et al. 1970; Ceron et al. 1995; FMC

1958, 1959a, 1980a; Gilbert and Mack 1995; Hoechst 1970, 1975, 1984e; Kiran and Varma 1988; Terziev et al. 1974). Convulsions have been observed after single oral doses of 10 mg/kg in dogs (FMC 1959a), after a single dose of 5 mg/kg in rats (Gilbert and Mack 1995), after a single dose of 15.1 mg/kg in rabbits (Ceron et al. 1995), after 3 daily doses of 2.5 mg/kg/day in dogs (FMC 1959a), after 10 daily doses of 1.8 mg/kg/day in pregnant rabbits (FMC 1981), and after 14 daily doses of 10 mg/kg/day in pregnant rats (FMC 1980a). At 2.5 mg/kg/day for 14 days, pregnant rats displayed poor muscle tone and head swaying (FMC 1980a); and at 6 mg/kg/day for 14 days, pregnant rats displayed face rubbing, flaccidity, and hyperactivity (FMC 1980b). Cerebral congestion and edema are often observed at necropsy in animals that die following acute ingestion of endosulfan (Boyd and Dobos 1969; Boyd et al. 1970; Terziev et al. 1974). A study designed to test anticonvulsants for their effectiveness in reducing the lethality of endosulfan found that phenobarbital, administered following administration of a lethal dose of endosulfan (80 mg/kg), significantly decreased the mortality and signs of neurotoxicity (e.g., convulsions and spasms) (Hoechst 1984e).

Some of the severe central nervous system effects described above have not been described in some intermediate or chronic ingestion studies of endosulfan in experimental animals (FMC 1959a, 1965, 1967; Hoechst 1984b, 1988b, 1989a). For example, in rats given daily gavage doses of 5 mg/kg/day (males) and 1.5 mg/kg/day (females) of endosulfan for 30 days, signs of central nervous system stimulation were observed for the first 3–4 days only and subsided thereafter (Dikshith et al. 1984). However, dogs that ingested feed containing 30 ppm for 54 days, 45 ppm for 52 days, and 60 ppm for up to 40 days (a TWA dose of 2.9 mg/kg/day for males or 2.6 mg/kg/day for females) showed extreme sensitivity to noise, frightened reactions to optical stimuli, and tonic contractions of the muscles of the extremities, face, and jaw (Hoechst 1989c). Animals exhibiting these symptoms were sacrificed to prevent needless suffering. Prior to sacrifice, the reflexes of these animals were tested. The placing and righting reflexes were absent, but pupillary, flexor, patellar, oral, and cutaneous reflexes were unaffected. At autopsy, results of routine gross and microscopic examination of the cerebral cortex, brain stem, cerebellum, medulla, optic and sciatic nerves, and spinal cord were normal. At slightly lower doses in this study, approximately 2.5–6 hours after consuming 2 mg/kg/day (males) or 1.8 mg/kg/day (females), dogs showed convulsive spasms of the jaws and abdominal muscles without vomiting. Pathology of the gastrointestinal tract did not reveal any adverse effects on these tissues, suggesting that the nervous system may have been the cause of the spasms. However, no effects on reflexes were observed, and gross and microscopic examination of central nervous system tissue revealed no abnormalities. Thus, it is unclear whether the effects observed at this dose were centrally mediated or were responses to gastrointestinal disturbances. Increased brain weights were observed in female rats following consumption of doses of 4.59 mg/kg/day

for 13 weeks (Hoechst 1985a) and in F_0 parental females in a multigeneration reproduction study at doses of 0.75 mg/kg/day (Hoechst 1984a). However, similar results were not observed in other studies in rats at doses of 2.9 mg/kg/day (males) and 3.8 mg/kg/day (females) for up to 2 years (Hoechst 1989a) or in mice or dogs in intermediate- and chronic-duration studies (FMC 1959a, 1967; Hoechst 1984b, 1988b, 1989c). Thus, the significance of the increases in brain weight is unknown, but it could have been related to edema.

A series of experiments were conducted in male Long-Evans rats to (a) assess the generality of an increased and persistent susceptibility to seizures following endosulfan treatment, (b) test the bidirectionality of kindling transfer induced by chemical and electrical means, and (c) determine whether chemical kindling reflects cumulative endosulfan toxicity (Gilbert 1992; Gilbert and Mack 1995). The findings can be summarized as follows: (1) a single gavage dose of 2.5 mg/kg of endosulfan reduced the threshold for seizure activity by electrical stimulation in amygdala kindled rats; (2) previous electrical stimulation reduced the threshold for convulsions by a single endosulfan dose; (3) repeated pretreatment with endosulfan followed by a 2-week drug-free period reduced the threshold for seizures by a challenge dose of endosulfan, arguing against cumulative toxicity; and (4) repeated pretreatment with endosulfan reduced the threshold for seizures by electrical stimulation. The positive transfer to electrical kindling suggested a commonality in the mechanism between seizures induced by repeated administration of endosulfan and those produced by repeated electrical stimulation.

The effects of endosulfan on the concentration of neurotransmitter substances in various regions of the brain from rats has been examined (Lakshmana and Raju 1994). These authors found that, relative to controls, treatment of newborn rats by gavage with technical endosulfan (6 mg/kg) for 8 days resulted in changes (increases and decreases) in the levels of noradrenaline, dopamine, and serotonin in the areas of the central nervous system that were examined (olfactory bulb, hippocampus, visual cortex, brainstem, and cerebellum). Treatment for 23 days also resulted in changes in neurotransmitter levels, but either of different magnitude or different direction than those observed in the animals exposed for 8 days, indicating that duration of exposure is an important parameter to consider when dealing with very young animals. Lakshmana and Raju (1994) also conducted a behavioral test in the rats treated for 23 days and found that treated rats took 29% more time to learn a task than the matched controls. The neurobehavioral effects of endosulfan have also been examined by others. Treatment of immature male rats with 2 mg technical endosulfan/kg/day by gavage for 90 days resulted in inhibition of learning and memory processes, and increased spontaneous motor activity (Paul et al. 1994). Since motor coordination was not significantly altered, Paul et al. (1994) suggested that the impairment in memory and learning

was due to a motivation deficit rather than to motor impairment. The learning process, but not the memory process, was reinstated by a serotonin depletor, suggesting that endosulfan produced a learning deficit by increasing serotonergic activity. In a subsequent study by the same group of investigators, in which both male and female rats were tested, it was found that a 30-day treatment with endosulfan in the diet (3 mg/kg/day) increased spontaneous motor activity to a greater degree in males than in females, but there was no sex difference regarding the impairment in memory and learning processes (Paul et al. 1995). The authors (Paul et al. 1995) speculated that the more marked effect in males may have been due to males preferentially metabolizing endosulfan to a more lipophilic metabolite, endosulfan sulfate, which could have reached the central nervous system. However, other factors cannot be ruled out, in particular since based on the chemical properties described in Chapter 3, endosulfan sulfate does not appear to be significantly more lipophilic than the parent compound.

In summary, neurotoxic effects of endosulfan are usually apparent only after acute ingestion of relatively high doses. Cumulative neurotoxicity does not appear to be significant. If the animal survives the acute toxic effects, then no long-term neurotoxic effects are evident from behavioral, gross, and microscopic observations. However, some impairment may occur that can be detected only by specialized neurobehavioral testing.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive toxicity in humans after oral exposure to endosulfan.

Three studies examined the effects of endosulfan exposure on reproductive performance in rats. Consumption of estimated doses of endosulfan of up to 9 and 8 mg/kg/day by male and female rats, respectively, for 2 weeks prior to mating and continued consumption throughout gestation resulted in no adverse effect on mating performance, pregnancy rate, or gestation (Hoechst 1982). This study is limited in that the actual intake of test material was quantified only during the first 2 weeks of exposure, and a relatively small number of animals was used. Similarly, consumption of 5 mg/kg/day by male rats and 1.5 mg/kg/day by female rats for 30 days prior to mating had no adverse effects on fertility when the

matings of treated males with control females and treated females with control males were compared to the mating of control males and females (Dikshith et al. 1984). This study is limited in that only 5 males and 10 females per dose were tested. A two-generation reproduction study in rats detected no effect on the size, mortality, or sex ratio of the litters following consumption of doses as high as 3.75 mg/kg/day for 84 days prior to the F_0 mating and 98 days prior to the F_1 mating (Hoechst 1984a).

A number of studies that used dose levels comparable to those described above have not observed adverse effects on the reproductive organs of rats, mice, or dogs. For example, routine gross and histopathological examination of the reproductive organs of male and female rats that ingested doses of endosulfan of 5 mg/kg/day for 15 days or 2 years revealed no adverse effects on these organs (FMC 1959b; Gupta and Gupta 1977a; Hack et al. 1995; Hoechst 1989a). Similarly, routine gross and histopathological examination of the reproductive organs of mice that consumed doses of 7.3 mg/kg/day (males) and 7.52 mg/kg/day (females) for 13 weeks (Hoechst 1984b) or 2.51 mg/kg/day (males) and 2.86 mg/kg/day (females) for 2 years (Hack et al. 1995; Hoechst 1988b; NCI 1978) revealed no toxic effects. Also, routine gross and microscopic examination of the reproductive organs of dogs that consumed doses of 2.9 mg/kg/day (TWA dose; males) and 2.6 mg/kg/day (TWA dose; females) for 146 days (Hoechst 1989c) or 2 mg/kg/day (males) and 1.8 mg/kg/day (females) for 1 or 2 years showed no adverse effects (FMC 1959a, 1967; Hoechst 1989c).

Other studies that conducted a more detailed examination of the reproductive organs of male animals have reported adverse reproductive effects. Reduced sperm count and altered testicular enzyme activities, indicating altered spermatogenesis, were reported in mature rats treated by gavage with 2.5 mg technical endosulfan/kg/day (the lowest dose tested), 5 days/week for 70 days (Sinha et al. 1995). Additional effects seen at higher doses (5 and 10 mg/kg/day) included reduced intratesticular spermatid count and daily sperm production, and increased incidence of abnormal sperm. All of these effects were also observed in young male rats (3 weeks old) treated by gavage with 2.5 mg technical endosulfan/kg/day (the lowest dose tested), 5 days/week for 90 days, suggesting that the younger animals were more sensitive than the older ones (Sinha et al. 1997). Altered spermatogenesis was also reported in male mice treated by gavage with 3 mg technical endosulfan/kg/day for 35 days (Khan and Sinha 1996). Similar results had been observed in earlier studies that tested higher doses of endosulfan. For example, male rats given oral doses of 10 mg/kg/day of endosulfan for 15 days had decreased weight of the testes with marked degenerative changes in the epithelium of the seminiferous tubules (Gupta and Chandra 1977). A limitation of the study is that high mortality of males was observed at 10 mg/kg/day. Similarly, male rats that consumed a TWA dose of 47.6 mg/kg/day for up to 74 weeks had testicular atrophy with

degeneration and necrosis of germinal cells lining the seminiferous tubules, multinucleated cells, and calcium deposition resulting in aspermatogenesis (NCI 1978; Reuber, 1981). This study is also limited due to the high mortality from kidney disease observed among the males at this dose.

Others reported effects on testosterone production in male rats after exposure to endosulfan doses in the range of 7.5–10 mg/kg/day, which may possibly lead to reproductive toxicity Singh and Pandey (1989). However, the results of this study were highly variable and frequently not dose related, making interpretation of the significance of the results difficult. A subsequent study (Singh and Pandey 1990) indicated a dose-related decrease in testicular testosterone, plasma testosterone, LH, and FSH in groups of male Wistar rats administered endosulfan at 0, 7.5, or 10 mg/kg/day for 15 or 30 days. Testicular microsomal cytochrome P450-dependent monooxygenases were also significantly inhibited at both dose levels after 30 days of exposure. All of the effects from 30 days of exposure were reversible during a 7-day recovery period, except for testicular testosterone, which remained depressed; no recovery period was utilized for the 15-day exposures. Singh and Pandey (1990) observed no significant effect on testis wet weight after 15 or 30 days of endosulfan administration at 7.5 or 15 mg/kg/day, while increased relative testes weight was observed following ingestion of 5 mg/kg/day by male rats for 30 days (Dikshith et al. 1984). However, the study is limited in that only five male rats were tested per dose.

In summary, although the available reproductive studies indicate endosulfan has no adverse effects on reproductive performance in animals, adverse effects on male reproductive organs have been seen in young rats and mice. The lack of effects seen in the studies that examined reproductive performance (specifically fertility rate) in treated males and females seems difficult to explain, given the finding of altered spermatogenesis in the more recent studies.

The highest NOAEL values and all reliable LOAEL values for reproductive effects in rats, mice, and dogs for each duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No clear geographic association was observed between the level of pesticide use and the locations of homes of children who underwent surgical correction for cryptorchidism (failure of descent of testes) in the Granada region of Spain (Garcia-Rodriguez et al. 1996). Endosulfan exposure levels were unavailable, but another study reported endosulfan isomers and/or metabolites in adipose tissue of 20 of

50 children (40%) who were hospitalized in the Granada hospital for a variety of reasons (Olea et al. 1999), indicating that significant endosulfan exposures occurred in the region.

Developmental effects have been observed in rats following oral administration of endosulfan to pregnant dams during gestation. Daily administration of endosulfan at doses of 5 or 10 mg/kg/day during Gd 6–14 produced a statistically significant increase in the percentage of resorptions and skeletal variations in the fetuses (e.g., absent fifth sternebrae) (Gupta et al. 1978). A dose-related increase in maternal deaths was observed in both test groups. Thus, embryotoxic effects were observed at doses that also caused maternal toxicity. This study is limited in that dosing was not continued until day 15 and, therefore, did not include the entire period of organogenesis. No statistically significant effect on fetal weight, sex ratio, or skeletal, internal, or external development was observed following administration of doses of 1.5 mg/kg/day to pregnant rats during Gd 6–15 (FMC 1972). A slight increase in the incidence of nonossified sternebrae was observed but did not reach statistical significance. Statistically significant skeletal variations (e.g., bipartite and misaligned sternebrae) were observed in fetuses following daily administration of doses of 0.66 mg/kg/day to pregnant rats during Gd 6–19 (FMC 1980b). These variations did not, however, increase with dose, and the incidence observed at the highest dose tested (6 mg/kg/day) did not reach statistical significance. Thus, these effects cannot be used to set the LOAEL for the study. At 6 mg/kg/day, additional fetal toxicity was observed (e.g., decreased fetal weight and length and other skeletal variations). Therefore, 6 mg/kg/day was set as the LOAEL for developmental toxicity in this study. However, the observation of statistically significant changes at lower doses places some uncertainty on this LOAEL. Maternal toxicity (e.g., two deaths, decreased mean corrected body weight gain measured on Gd 20, face rubbing, flaccidity, and hyperactivity) was observed in the high-dose group (6 mg/kg/day) and to a lesser extent in the mid-dose group (2 mg/kg/day) (e.g., decreased mean corrected body weight gain measured on Gd 20 and face rubbing). Limitations of this study include a number of gavage errors and the unplanned addition of 10 more animals to the high-dose group and 5 more animals to the control group (mated at approximately 30 and 40 days after the initial mating). A range-finding study detected no developmental toxicity at doses as high as 10 mg/kg/day (FMC 1980a). However, this study used a small number of animals and effects on implantation and resorption were the only developmental end points examined.

Exposure of rabbits to endosulfan during Gd 6–28 produced no significant effects on the number of implants, litter size, sex ratio, fetal weight or length, or the percentage of live or resorbed fetuses at doses as high as 1.8 mg/kg/day (FMC 1981). However, dams treated with 1.8 mg/kg/day did exhibit neurotoxic signs (e.g., noisy and rapid breathing, hyperactivity, and convulsions) that were considered to be

treatment related. Such neurotoxic effects were not observed at 0.7 mg/kg/day. Animals from control groups, as well as all test groups developed ascites, and 6 rabbits were added to the 1.8-mg/kg/day dose group without concurrent controls. The occurrence of ascites in both control and treated animals indicates that a problem existed in the laboratory environment, and thus casts doubt on the credibility of the results from the FMC (1981) study.

In addition to the studies noted above that examined the effect of endosulfan administered only during the period of gestation, two studies have examined the effects of endosulfan on fetal development following administration prior to mating, as well as throughout gestation and lactation. Administration of endosulfan at doses as low as 6 mg/kg/day from 2 weeks prior to mating through weaning resulted in a significant decrease in mean litter weight during lactation (Hoechst 1982). At 8 mg/kg/day in this study, an increase in pup mortality was also observed. Maternal toxicity (e.g., decreased body weight and increased relative liver weight) was observed in females at 6 mg/kg/day and above. In the second study, consumption of 0.75 mg/kg/day and 3.75 mg/kg/day of endosulfan for 84 days prior to mating through weaning resulted in decreased litter weights of rats during lactation (Hoechst 1984a). At 3.75 mg/kg/day, increases in pituitary weights and uterine weights were also observed among the weanlings. Maternal toxicity (e.g., decreased body weight) was observed at 3.75 mg/kg/day. Both of these studies are limited in that insufficient information was provided regarding the intake of test material during gestation and lactation.

In summary, based on these studies, the evidence for endosulfan-induced adverse developmental effects in animals is inconclusive. The highest NOAEL value and all reliable LOAEL values for developmental effects in rats and rabbits for the acute- and intermediate-duration categories are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to endosulfan.

Genotoxicity studies in animals following oral exposure to endosulfan have yielded both positive and negative results. In male rats, acute exposure to doses of up to 22 mg/kg/day of endosulfan for 5 days did not induce chromosomal aberrations in either bone marrow (somatic) or spermatogonial (germinal) cells. The ratio of mitotic index and frequency of chromatid breaks in the two cell types had no correlation with the doses tested and were not significantly different from the control group (Dikshith and Datta 1978). In

mice, a statistically significant increase in chromosomal aberrations was observed 60 days after initial treatment with oral doses of 6.4 mg/kg/day of endosulfan for 5 days (Usha Rani and Reddy 1986). However, mice fed 21.7 mg/kg/day for 2 days did not show a statistically significant increase in the frequency of micronuclei in bone marrow erythrocytes 6 hours posttreatment (Usha Rani et al. 1980).

Oral administration of 11.6 mg/kg/day of endosulfan to rats for up to 30 days also failed to induce chromosomal damage in bone marrow and spermatogonial cell systems, but it is not known how soon after treatment the animals were killed. As shown in mouse studies (Usha Rani and Reddy 1986), a latency period of 60 days was required to see chromosomal aberrations in spermatogonia. However, relatively significant changes were observed for mitotic indices (Dikshith et al. 1978).

In summary, endosulfan was not shown to be genotoxic following oral exposure of rats, but the data are inconclusive. It induces chromosomal aberrations and gene mutations in mice and *Drosophila*. Further complicating analysis of this data, is the possibility that formulations of endosulfan used in these studies may have contained epichlorohydrin, a well documented genotoxic chemical, as a stabilizer (Hoechst 1990). Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to endosulfan. Carcinogenic effects of endosulfan were investigated in a number of chronic animal bioassays with rats and mice; the available data provide no evidence that endosulfan is carcinogenic.

Carcinogenicity in rats was first assayed in Osborne-Mendel rats by NCI (1978). The assay was flawed because the female rats were given endosulfan for less than their entire lifetime (78 out of 110 weeks); high early mortality in the males caused the high- and low-dose males to be terminated at 74 and 82 weeks, respectively, while half of the control males continued on study until 110 weeks; and the doses were changed several times during the study. The poor survival in the male rats precluded drawing a conclusion regarding the carcinogenicity of endosulfan in males because insufficient numbers of animals were alive to demonstrate a risk from late-developing tumors. However, the authors concluded that under the conditions of the assay, endosulfan was not carcinogenic in female rats.

Histological sections from this study were reevaluated by Reuber (1981) who concluded that endosulfan was carcinogenic. By grouping tumors, Reuber identified statistically significant increases in the total

number of malignant tumors in both high-dose females (TWA dose, 22.3 mg/kg/day) and low-dose females (TWA dose, 11.1 mg/kg/day), as well as in the total number of carcinomas and sarcomas in high-dose females and lymphosarcomas in high-dose males (TWA dose, 47.6 mg/kg/day) and high-dose females. No increases in tumor incidence were identified in any specific tissue, and Reuber's conclusions were not independently confirmed by other scientists.

The carcinogenicity of technical endosulfan was reevaluated in Sprague-Dawley rats using lower doses of endosulfan (Hoechst 1989a). Endosulfan was administered in the diet for 2 years, and no effect on survival was observed in either sex at any dose. Under the conditions of this assay, dietary consumption of doses as high as 3.8 mg/kg/day by females or 2.9 mg/kg/day by males did not result in an increase in the incidence of any neoplastic lesions in these animals. The results from the Hoechst (1989a) bioassay were subsequently published in the open literature (Hack et al. 1995). In an additional study, no increase in neoplastic lesions was observed in Wistar rats that consumed doses of endosulfan as high as 5 mg/kg/day (males) or 1.5 mg/kg/day (females) for 2 years (FMC 1959b). However, this study is limited in that relatively few rats were used (25/sex/dose), and histopathological evaluation was limited to 5 rats/sex/dose plus any grossly observed lesions.

Carcinogenicity has also been evaluated by NCI using mice (NCI 1968, 1978). Two strains of mice ($B6C3F_1$ and $B6AKF_1$) were tested in the 1968 study. The $B6C3F_1$ mice are the product of mating C57BL/6 females with C3H/Anf males. The $B6AKF_1$ mice are the product of mating C57BL/6 females with AKR males. These hybrids were used because their susceptibility to carcinogenic stimuli was expected to be high. Each treatment group, and each vehicle, positive and negative control group consisted of 18 males and females of each strain. The animals were administered endosulfan in 0.5% gelatin daily by gavage at doses of 1.0 and 2.15 mg/kg/day from 7 to 28 days after birth. From 28 days to 18 months they were given endosulfan in the diet *ad libitum*. Concentrations in the diet were calculated so that the endosulfan doses consumed by the animals were the same as those given by gavage. However, these calculations were based on starting body weights, and no adjustments were made to account for growth and changes in food intake throughout the 18-month exposure period. A statistically significant increase ($p<0.05$) in the incidence of total tumors and pulmonary adenomas was reported for endosulfan; it appears, however, that the data for both strains and doses were combined to perform these statistical analyses. Therefore, it is not possible to assess the validity of these conclusions. Furthermore, the summary data sheets do not clearly indicate the dose, so it appears that the dose level with low survival was not the same dose level that displayed an increase in tumor incidence. Because of the incomplete

reporting and the confusion regarding statistical analysis of tumor incidence data, these results cannot be considered adequate evidence of the carcinogenicity of endosulfan.

Carcinogenicity was reassayed by NCI (1978) in B6C3F_1 mice. Fifty mice per sex per dose were used. Male mice were given 0, 0.46, or 0.9 mg/kg/day (TWA doses). Female mice were given 0, 0.26, or 0.5 mg/kg/day for 78 weeks. Then mice of both sexes were observed for an additional 14 weeks. No statistically significant increases in tumor incidence were observed in female mice. Because mortality in male mice was high in all groups, a conclusion regarding carcinogenicity in males was not made. Reuber (1981) also reevaluated histological sections from this study and concluded that a significant increase in the incidence of hepatic carcinomas occurred in the low-dose female mice. However, he indicated that histological sections from the liver were inadequate, and his conclusions were not independently verified by other scientists.

The carcinogenicity of technical endosulfan was also evaluated in NMRI mice exposed through the diet to endosulfan for 2 years at doses as high as 2.51 mg/kg/day in males and 2.86 mg/kg/day in females (Hoechst 1988b). Sixty mice/sex/dose were used. No increase in the incidence of any neoplastic lesion was identified in either males or females at any dose. These results were later published in the open literature (Hack et al. 1995).

The ability of technical endosulfan (98.8% pure), α-endosulfan, and β-endosulfan to act as tumor promoters in a two-stage, altered hepatic foci bioassay was examined in male Sprague-Dawley rats (Fransson-Steen et al. 1992). The animals were initiated by intraperitoneal injection of nitroso-diethylamine followed by 2/3 partial hepatectomy. Five weeks later, they were transferred to a diet that provided approximately 1.5, 5, or 15 mg test material/kg/day for 20 weeks. Promoting activity was evaluated for the development of foci of gamma-glutamyltranspeptidase-positive hepatocytes (AHF). Of the three chemicals tested, the α-isomer exhibited the strongest promoting activity; in initiated rats it caused a significant and dose-related increase in both the volume fraction of liver occupied by AHF and the number of AHF/cm^3, and only the highest dose increased the mean foci volume. Technical endosulfan and the β-isomer increased the volume fraction of liver occupied by AHF and the number of AHF/cm^3, but the responses were not dose-related, and neither increased mean foci volume. Endosulfan, the α-isomer, and the β-isomer induced no or few AHF in rats that were not initiated.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to endosulfan. However, dermal exposure to endosulfan caused death in livestock and experimental animals. Nicholson and Cooper (1977) described the case of five calves that were "dusted liberally" in the late afternoon with endosulfan to remove lice. The dose was not specified. By 7:00 a.m. the next morning, one calf was dead, and the remaining four calves displayed signs of neurotoxicity: muscle tremors, twitching of the ears, snapping of the eyelids, hyperactivity, and tonic-clonic convulsions. By the end of the day, three more calves died, and the remaining calf recovered without complications. A necropsy performed on one of the calves revealed no gross lesions.

Lethality data from studies using experimental animals indicate that the lethal dose varies substantially depending on the species and the sex of the animal tested. The dermal LD_{50} obtained following a single dermal application of endosulfan to the backs of female rabbits was in the range of 167–182 mg/kg of endosulfan (Gupta and Chandra 1975). However, 2 out of 3 female rats died following exposure to 31.25 mg/kg/day β-endosulfan for 6 hours/day for 5 days (Hoechst 1989b). In contrast, exposure to 250 mg/kg/day during the same 5-day period was not lethal to male rats. At 500 mg/kg/day, 2 of 3 males died. This study is limited, however, by the small number of animals tested. Single dermal doses of 1,500 or 2,250 mg/kg applied to clipped skin of pregnant rats (number per exposure group was not clearly reported) on gestation day 1 resulted in death of at least two females, but no maternal deaths were reported at #1,000 mg/kg (EI Dupont deNemours & Co. 1973).

Similar differences in lethality were observed between different sexes and species during slightly longer exposure periods (Hoechst 1985c, 1985d). Exposure for a total of 21 days out of 30 for 6 hours/day, 5 days/week, resulted in deaths in males treated with doses of 81 mg/kg/day and in females treated with 27 mg/kg/day (Hoechst 1985c). In contrast, female guinea pigs appeared to be relatively resistant to endosulfan toxicity (Hoechst 1983b). Only 1 female out of 20 died when exposed to 587 mg/kg/day, for 6 hours/day, 3 days/week for 3 weeks, and it was unclear whether this death was treatment related. In the majority of these reports, the clinical signs observed prior to death (tremors, salivation, and convulsions) were similar to those seen following oral exposure to endosulfan (see Section 2.2.2.1).

All reliable LD_{50} and LOAEL values for death in each species and duration category are recorded in Table 2-3. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

2.2.3.2 Systemic Effects

The primary systemic targets of endosulfan toxicity in animals following dermal exposure are the liver and kidney. Adverse hematological effects have also been observed following dermal administration of endosulfan. No studies were located regarding musculoskeletal effects in humans or animals after dermal exposure to endosulfan.

The highest NOAEL value and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-3. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

Respiratory Effects. Increased occurrence of dyspnea and increased respiratory rate were noted in 18 agricultural workers in India who applied endosulfan without protective equipment (both dermal and inhalation exposures probably occurred) (Chugh et al. 1998).

Dyspnea and decreased respiration were observed in female rabbits prior to death following a single dermal application of 225 mg/kg of endosulfan (Gupta and Chandra 1975). It is unclear whether similar effects were observed at lower doses in this study. Irregular respiration was also observed in male and female rats as the result of 5 daily, 6-hour/day exposures of β-endosulfan at doses of 16 mg/kg/day (females) and 250 mg/kg/day (males) (Hoechst 1989b). These doses were the highest doses at which no deaths were observed. Acute congestion of the lungs with dilation of alveolar capillaries was observed at necropsy of animals that died as the result of exposure to doses of 31.25 mg/kg/day and above (females) and 500 mg/kg/day and above (males) in this study. Congestion of the lungs was also observed at necropsy of rats dying as the result of a 30-day, 6-hour/day, 5-day/week exposures to endosulfan at 81 mg/kg/day (males) and 27 mg/kg/day (females) (Hoechst 1985c). It is probable that these effects are a result of generalized effects on central nervous system activity and attendant sequelae.

Table 2-3. Levels of Significant Exposure to Endosulfan - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE						
Death						
Rat (CD-1)	once Gd 1				1500 F (lethal dose)	El DuPont Denemours & Co. 1973
Rat (Wistar)	5 d 6 hr/d				31 F (2/3 died)	Hoechst 1989b Beta
Rat (Wistar)	5 d 6 hr/d				500 M (3/3 died)	Hoechst 1989b Beta
Rabbit (albino)	1 d				167 (LD_{50})	Gupta and Chandra 1975 Technical
Systemic						
Rat (Wistar)	5 d 6 hr/d	Resp		16 F (irregular respiration)	31 F (lungs filled with blood)	Hoechst 1989b Beta
		Gastro		16 F (diarrhea; mesenteric blood vessels distended)	31 F (small intestines filled with reddish fluid)	
		Hepatic		31 F (dark discoloration of the liver)		
		Dermal	31	62 F (slight to moderate erythema; slight edema)		
Rabbit (albino)	1 d	Hepatic			100 F (congestion; degeneration; necrosis)	Gupta and Chandra 1975 Technical
		Renal			100 F (shrunken glomerular tufts; necrosis of tubular epithelial cells)	
		Endocr			100 F (swollen adrenal cells with foamy cytoplasm and eccentric nuclei)	

Table 2-3. Levels of Significant Exposure to Endosulfan - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Rabbit	24 hr	Dermal		263 (mg/kg) (slight erythema)		Industria Prodotti Chimici 1975 Technical
Immunological/Lymphoreticular						
Rat (Wistar)	5 d 6 hr/d		500	1000 M (spleen reduced in size)		Hoechst 1989b Beta
Neurological						
Rat (Wistar)	5 d 6 hr/d				16 F (decreased activity; convulsions)	Hoechst 1989b Beta
Developmental						
Rat (CD-1)	once Gd 1		450		670 (exencephaly)	El DuPont Denemours & Co. 1973

Table 2-3. Levels of Significant Exposure to Endosulfan - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
				Less Serious (mg/kg/day)		Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Death							
Rat (Wistar)	30 d 5 d/wk 6 hr/d					27 F (5/6 died)	Hoechst 1985c Technical
Rat (Wistar)	30 d 5 d/wk 6 hr/d					81 M (3/6 died)	Hoechst 1985c Technical
Rat (Wistar)	30 d 5 d/wk 6 hr/d					48 F (4/11 died)	Hoechst 1985d Technical
Rat (Wistar)	30 d 5 d/wk 6 hr/d					192 M (2/11 died)	Hoechst 1985d Technical
Systemic							
Rat (Wistar)	30 d 1x/d	Hemato		19 M (decreased hemoglobin)			Dikshith et al. 1988 Technical
		Hepatic		10 F (decreased hepatic GOT and GPT; increase serum AP and LDH)			

Table 2-3. Levels of Significant Exposure to Endosulfan - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
				Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Rat (Wistar)	30 d 5 d/wk 6 hr/d	Resp	27 M		81 M	(acute lung congestion; dilation of alveolar vessels)	Hoechst 1985c Technical
		Cardio	27 M		81 M	(blood vessel congestion; cardiac ventricles filled with blood; acute heart and circulatory failure)	
		Gastro	81 M				
		Hemato	81 M				
		Musc/skel	81 M				
		Hepatic	81 M				
		Renal	81 M				
		Endocr	81 M				
		Dermal	81 M				
Rat (Wistar)	30 d 5 d/wk 6 hr/d	Resp	9		27 F	(acute lung congestion; dilation of alveolar vessels)	Hoechst 1985c Technical
		Cardio	9		27 F	(blood vessel congestion; cardiac ventricles filled with blood)	
		Gastro	27				
		Hemato	27				
		Hepatic	27				
		Renal	27				
		Endocr	27				
		Dermal	27				
Rat (Wistar)	30 d 5 d/wk 6 hr/d	Hemato	48	192 M	(elevated serum protein; decreased serum cholinesterase)		Hoechst 1985d Technical
		Hepatic	48				
		Renal	48				
		Dermal	48				

Table 2-3. Levels of Significant Exposure to Endosulfan - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Gn pig (Pirbright-White)	3 wk 3 d/wk 6 hr/d	Dermal	587 (mg/kg) F			Hoechst 1983b Technical
Immunological/Lymphoreticular						
Gn pig (Pirbright-White)	3 wk 3 d/wk 6 hr/d		587 (mg/kg) F			Hoechst 1983b Technical
Neurological						
Rat (Wistar)	30 d 5 d/wk 6 hr/d			1 F (decreased brain cholinesterase)	81 (convulsions; diffuse brain edema)	Hoechst 1985c Technical
Rat (Wistar)	30 d 5 d/wk 6 hr/d			12 F (piloerection; slight lacrimation)	48 F (hypersalivation, tremors, convulsions)	Hoechst 1985d Technical

AP = alkaline phosphatase; ATPase = adenosine triphosphatase; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gn pig = guinea pig; GOT = glutamic-oxaloacetic transaminase; GPT = glutamic-pyruvic transaminase; Hemato = hematological; hr = hour(s); LDH = lactate dehydrogenase; LOAEL = lowest-observable-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; wk = week(s); x = times

Cardiovascular Effects. Both tachycardia and bradycardia were noted among 18 agricultural workers in India who applied endosulfan without protective equipment (both dermal and inhalation exposures probably occurred) (Chugh et al. 1998).

Blood vessels were congested and cardiac ventricles were distended with blood in rats that died as the result of a 6-hour/day, 5-day/week for 30-day exposure to 81 mg/kg/day (males) and 27 mg/kg/day (females) (Hoechst 1985c). However, it is unclear whether these effects were due to a direct action of endosulfan on the blood vessels and heart or were a result of a more general toxic insult (e.g., convulsions). The respective NOAELs for males and females were 27 and 9 mg/kg/day.

Gastrointestinal Effects. Abdominal discomfort after meals, nausea, and vomiting were noted in 18 agricultural workers in India who applied endosulfan without protective equipment (both dermal and inhalation exposures probably occurred) (Chugh et al. 1998). Another study reports gastrointestinal effects in 22 cases of acute poisoning of subjects spraying cotton and rice fields (Singh et al. 1992). Nausea, vomiting, pain in the abdomen, and diarrhea were among the signs and symptoms observed. Singh et al. (1992) assumed that exposure was mainly by the dermal route since subjects who sprayed the rice fields, and who suffered cuts over the legs with the sharp leaves of the rice plants exhibited the more severe toxicity.

Diarrhea was observed in rats exposed for 5 days, 6 hours/day to both lethal and sublethal doses of β-endosulfan ($250 mg/kg/day for males and $16 mg/kg/day for females) (Hoechst 1989b). Autopsy of animals from this study revealed that the mesenteric blood vessels of one of the surviving females exposed to 16 mg/kg/day were distended with blood, and that the small intestines of animals dying as a result of exposure were filled with a reddish fluid (500 mg/kg/day for males and 31.25 for mg/kg/day females). In contrast, no treatment-related effects were revealed by routine gross and histopathological examination of gastrointestinal tissues (stomach, small and large intestines, and pancreas) from rats exposed to doses of 27 mg/kg/day (females) and 81 mg/kg/day (males) for 30 days, 6 hours/day, 5 days/week (Hoechst 1985c).

Hematological Effects. Normal hemoglobin, hematocrit, white blood cell count, and differential and sedimentation rate were observed in a 35-year-old agricultural pilot approximately 8 hours after a 45-minute dermal exposure (with presumed concurrent inhalation exposure) when his clothing became soaked in endosulfan and methomyl (Cable and Doherty 1999).

Mixed results have been obtained in studies examining hematological effects of dermal exposure to endosulfan in rats. Although decreased hemoglobin was observed in male rats following daily application of doses of endosulfan of 18.75 mg/kg for 30 days (Dikshith et al. 1988), similar results have not been observed in female rats or in male rats at similar doses in other studies. For example, no hematological parameters were adversely affected following exposure of females to doses of 32 mg/kg/day for 30 days (Dikshith et al. 1988). In addition, no adverse effects on routine hematological parameters were observed following exposure of rats for 30 days for 6 hours/day, 5 days/week to doses of endosulfan ranging from 12 to 192 mg/kg/day (males) and from 3 to 48 mg/kg/day (females) (Hoechst 1985d). Similarly negative results were obtained in a comparable 30-day rat study using slightly lower endosulfan doses (Hoechst 1985c). Wistar rats were used in the studies by Dikshith et al. (1988) and Hoechst (1985c, 1985d); thus, the reason for the different results of these studies is unclear but may have been related to differences in the age of the rats or the application protocol.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after dermal exposure to endosulfan.

Hepatic Effects. Normal serum liver function tests (unspecified) were observed in a 35-year-old agricultural pilot approximately 8 hours after a 45-minute dermal exposure (with presumed concurrent inhalation exposure) when his clothing became soaked in endosulfan and methomyl (Cable and Doherty 1999).

Distinct hepatotoxicity has been observed in animal studies following acute-duration exposure to large dermal doses of endosulfan. The livers of female rabbits that survived a single dermal application of 100 mg/kg of endosulfan exhibited microscopic evidence of congestion, dilation of sinusoids, hepatocellular degeneration, hyperplastic Kupffer cells, focal necrosis, and portal tract and bile duct proliferation (Gupta and Chandra 1975). In addition, necropsy of rats that died following exposure for 5 days, 6 hours/day, to doses of endosulfan greater than or equal to 250 mg/kg/day (males) and 31.25 mg/kg/day (females) revealed darkly discolored livers (Hoechst 1989b).

Subchronic dermal exposures to slightly lower doses of endosulfan have been associated with more mild toxicity and adaptive changes. For example, histopathological examination of livers from male and female rats exposed to doses of 9 mg/kg/day 6 hours/day, 5 days/week for 30 days revealed slight fatty changes and an increased incidence of cellular hypertrophy and division (Hoechst 1985c). Similar changes were not observed in a repeat 30-day study at 12 or 192 mg/kg/day in males or at 48 mg/kg/day

in females (Hoechst 1985d). However, dermal administration of 18.75 mg/kg/day of endosulfan to male rats for 30 days resulted in decreases in hepatic levels of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase and increases in serum levels of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and alkaline phosphatase, but no changes in relative organ/body weight or other gross or histopathological evidence of liver damage (Dikshith et al. 1988). Effects in female rats at doses between 9.83 and 32 mg/kg/day were limited to decreases in hepatic glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase and increases in protein, hepatic alkaline phosphatase, and lactate dehydrogenase. The hepatic effects of long-term dermal exposure to endosulfan cannot be evaluated because of lack of data.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to endosulfan.

The kidneys of female rabbits given a single dermal application of 100 mg/kg of endosulfan exhibited shrunken glomerular tufts, thickened Bowman's capsules, and necrosis of the tubular epithelial cells (Gupta and Chandra 1975). However, daily application of up to 192 mg/kg/day (males) or 48 mg/kg/day (females) to the skin of rats for 30 days had no effect on kidney weight or histopathology (Dikshith et al. 1988; Hoechst 1985c, 1985d). The discrepancy may reflect species differences and/or differences in the application vehicle. The renal effects of long-term dermal exposure to endosulfan cannot be evaluated because of lack of data.

Endocrine Effects. No studies were located regarding endocrine effects in humans after dermal exposure to endosulfan.

The adrenals of rabbits given a single dermal dose of 100 mg/kg of endosulfan exhibited microscopic changes, including swollen cells with foamy cytoplasm and eccentric nuclei (Gupta and Chandra 1975). Also, release of lipids from the adrenal cortex was observed in rats that died following daily application of 81 mg/kg/day (males) and 27 mg/kg/day (females) to the skin for 6 hours/day, 5 days/week for 30 days (Hoechst 1985c). However, daily application of up to 62.5 mg/kg/day (males) or 32 mg/kg/day (females) of endosulfan to the skin of rats for 30 days had no effect on adrenal weight or histopathology (Dikshith et al. 1988).

Dermal Effects. No studies were located regarding dermal effects in humans after dermal exposure to endosulfan.

Mild dermal irritation has been observed in two studies following application of highly toxic amounts of endosulfan to the skin. A single 24-hour exposure of the skin of rabbits to 263 mg/kg of endosulfan resulted in only slight erythema (Industria Prodotti Chimici 1975). Daily application of β-endosulfan to rat skin for 6 hours/day for 5 days resulted in slight-to-moderate erythema, slight edema, and dry, rough, and scaling skin at 62.5 mg/kg/day in females and at 250 mg/kg/day in males (Hoechst 1989b). However, daily application of up to 48 mg/kg/day (females) or 192 mg/kg/day (males) to the skin of rats for 30 days (5 days/week, 6 hours/day) caused no apparent skin irritation (Hoechst 1985c, 1985d). Dermal application of 587 mg/kg/day of endosulfan 3 days/week, 6 hours/day for 3 weeks caused no erythema or edema in guinea pigs (Hoechst 1983b).

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to endosulfan.

Limited information was available regarding ocular irritation by endosulfan. An unspecified amount of a 20% aqueous suspension of endosulfan instilled in the eyes of rabbits did not produce any ocular irritation or congestion (Gupta and Chandra 1975).

2.2.3.3 Immunological and Lymphoreticular Effects

The only study located regarding immunological effects in humans after dermal exposure to endosulfan was an account of the results of patch tests on the backs of 14 farm workers with work-related dermatitis and 8 controls who were not exposed to pesticides (Schuman and Dobson 1985). Skin sensitization was not observed in any of the subjects following a 48-hour, closed-patch exposure to an unspecified amount of 0.1% endosulfan in petrolatum.

Extremely limited information was available regarding immunological effects of endosulfan in animals following dermal exposures. No sensitization was observed after a challenge application of 587 mg/kg/day to female guinea pigs 16 days following a 6-hour/day, 3-day/week, 3-week exposure to this dose (Hoechst 1983b). In addition, no effect on thymus weight was reported following a 30-day (6 hours/day, 5 days/week) exposure to concentrations of up to 81 mg/kg/day in males and 27 mg/kg/day in females (Hoechst 1985c). However, one of the male rats that died following exposure to 1,000 mg/kg/day, 6 hours/day for 5 days had a spleen that was reduced in size (Hoechst 1989b); however, it is unclear whether the reduction in size was an immunotoxic effect or due to some other more generalized toxic insult.

The highest NOAEL and all reliable LOAEL values for immunological effects in rats or guinea pigs following acute- or intermediate-duration dermal exposures are recorded in Table 2-3. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

2.2.3.4 Neurological Effects

As indicated in the section on inhalation exposure, neurotoxicity is the primary effect observed in humans following occupational exposure to endosulfan. Since dermal exposures may comprise a substantial portion of occupational exposure to endosulfan, the results presented in Section 2.2.1.4 are repeated here, along with reports of human exposures that were primarily dermal. Convulsions were reported in nine individuals exposed to the endosulfan-containing insecticide, Thiodan®, during bagging (Ely et al. 1967). In addition, a case of long-term, possibly permanent brain damage in an industrial worker was attributed by Aleksandrowicz (1979) to endosulfan exposure. This worker was exposed while cleaning vats that contained residues of endosulfan solution. The acute phase of the poisoning was manifested by repeated convulsions and impaired consciousness. After recovery, the patient became disoriented and agitated. Two years later, he exhibited cognitive and emotional deterioration, memory impairment, and impairment of visual-motor coordination manifested by an inability to perform small tasks. However, modest alcohol consumption (1L of wine consumed per week) may have been a contributing factor directly on the brain or by decreasing metabolism of endosulfan in the liver. A 35-year-old male agricultural pilot experienced nausea, weakness, coldness, and blurred vision after 30 continuous minutes of dermal exposure when his clothes became soaked in endosulfan and methomyl, and tonic-clonic seizures 6 hours after a total of 45 minutes of dermal exposure (with presumed concurrent inhalation exposure) (Cable and Doherty 1999). Serum cholinesterase was within the normal range at 30 hours postexposure. A computed tomography (CT) scan showed no abnormalities, and the patient was discharged after 2 days of neurological observation, but three serial outpatient electroencephalographs (EEGs) showed a persistent nonspecific epileptic focus in the cerebral frontal lobes. In another study, dizziness, nausea, confusion and irritability, muscle twitching, tonic/clonic convulsions, and conduction defects were noted in 18 agricultural workers in India who applied endosulfan without protective equipment (both dermal and inhalation exposures probably occurred) (Chugh et al. 1998). Limitations associated with these reports include lack of quantitative exposure data, lack of data on the duration of exposure, the possibility of multiple routes of exposure (i.e., oral and dermal as well as inhalation), and possible concurrent exposure to other chemicals. Therefore, this information can only provide qualitative evidence of neurotoxicity associated with dermal exposure to endosulfan in humans.

Twenty-two cases of endosulfan poisoning were reported in people exposed while spraying cotton and rice fields; the dermal route of exposure was assumed to be the primary route of exposure (Singh et al. 1992). The assumption was based on the fact that those spraying rice fields, and who suffered cuts over the legs with the sharp leaves on the rice plants exhibited the more severe toxicity. Three out of the 22 cases exhibited tremors and 11 presented convulsions; all patients recovered.

Central nervous system stimulation similar to that reported for occupational exposure is seen following acute dermal exposure to endosulfan in experimental animals. The spectrum of effects includes hyperexcitability, tremors, decreased respiration, tonic-clonic convulsions, and ultimately death (Gupta and Chandra 1975; Hoechst 1989b; Nicholson and Cooper 1977). In rats, the lowest doses associated with these effects were 16 mg/kg/day in females and 250 mg/kg/day in males during a 6-hour/day, 5-day exposure regimen (Hoechst 1989b).

Similar signs of central nervous system stimulation were observed following exposure to doses of endosulfan as low as 48 mg/kg/day (females) and 81 mg/kg/day (males) during a 6-hour/day, 5-day/week, 30-day exposure period (Hoechst 1985c, 1985d). Diffuse edema was also observed in the brains of males at the 81-mg/kg/day exposure level. However, daily application of up to 62.5 mg/kg/day (males) or 32 mg/kg/day (females) of endosulfan to the skin of rats for 30 days had no effect on brain weight or histopathology (Dikshith et al. 1988). No information was found regarding neurological effects of long-term dermal exposure to endosulfan.

The highest NOAEL and all reliable LOAEL values for neurological effects in rats following acute- and intermediate-duration dermal exposures are recorded in Table 2-3. In some studies, only the α- or β-isomer of endosulfan was tested. In such cases, a notation regarding the specific isomer tested is included in the effect description.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to endosulfan.

Limited information was available regarding reproductive effects in animals following dermal exposures to endosulfan. No effects on the reproductive organs were observed during routine gross and histopathological examination following exposure of rats to doses of 81 mg/kg/day (males) or 27 mg/kg/day (females) for 6 hours/day, 5 days/week for 30 days (Hoechst 1985c). Also, daily application of up to

62.5 mg/kg/day (males) or 32 mg/kg/day (females) to the skin of rats for 30 days had no effect on reproductive organ histopathology (Dikshith et al. 1988).

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans after dermal exposure to endosulfan.

An unspecified number of pregnant rats was administered a single dermal dose of 670 or 1,000 mg endosulfan/kg on clipped skin on gestation day 1, and exencephaly was observed in 5 and 3 pups, respectively (the total number of live pups at these dose levels was not clearly indicated) (EI Dupont deNemours & Co. 1973). Maternal death was reported at higher dose levels (1500 and 2250 mg/kg), no effects were reported at lower dose levels (0 and 450 mg/kg), and no increase in embryolethality was observed at any dose level. Further study details were not provided.

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to endosulfan.

2.3 TOXICOKINETICS

Data regarding toxicokinetics of endosulfan in humans are limited to information from cases of accidental or intentional ingestion of the chemical and cases of occupational exposure in the workplace where inhalation and/or dermal contact may have occurred. The evidence that humans absorb endosulfan by the inhalation and/or dermal routes of exposure is only indirect. Conclusive evidence exists regarding absorption through the gastrointestinal tract, although the extent of absorption is not known. Animals absorb endosulfan by the inhalation, oral, and dermal routes of exposure. Near 80% of the administered oral dose may be absorbed and near 20% or a dermal dose may be absorbed; the role of the administration vehicle has not been studied. Autopsy data in humans suggest that endosulfan may accumulate in the liver, kidney, and brain at least in the short-term. Data in animals also suggest that following initial distribution to adipose tissue, endosulfan accumulates in liver and kidney, and that the α-isomer

accumulates to a greater extent than the β-isomer. There is no information on the metabolism of endosulfan in humans. In animals, endosulfan is metabolized predominantly to polar and nonpolar metabolites by microsomal enzymes. Endosulfan and metabolites have been detected in the urine of humans after ingestion of the chemical. In animals, the feces is the main route of excretion of unchanged parent compound and metabolites. A physiologically based pharmacokinetic (PBPK) model for endosulfan has not been developed.

2.3.1 Absorption

Health effects in humans and animals provide indirect evidence of absorption of endosulfan following oral, inhalation, and dermal exposures. Endosulfan and metabolites have been detected in tissues of humans and animals following various exposures to endosulfan, providing qualitative evidence that endosulfan is absorbed. Endosulfan residues were found in fat of hospitalized Spanish children, indicating that absorption occurs in children (Olea et al. 1999), but no studies were located regarding known or suspected differences between children and adults with respect to endosulfan absorption.

2.3.1.1 Inhalation Exposure

No studies were located regarding the absorption of endosulfan following inhalation in humans and animals. However, Ely et al. (1967) described nine case reports of occupational exposure to endosulfan resulting in neurological effects. Also, neurological effects have been observed in rats following inhalation exposure to endosulfan (Hoechst 1983a). These studies describing the occurrence of neurotoxicity following inhalation exposure to endosulfan provide indirect evidence that endosulfan is absorbed by both humans and animals by this route of exposure.

2.3.1.2 Oral Exposure

Although no specific studies were located that quantified the absorption of endosulfan in animals or humans, indirect evidence of toxic effects following ingestion of endosulfan suggests that the gastrointestinal tract is a site of endosulfan absorption in humans and animals (Blanco-Coronado et al. 1992; Lo et al. 1995; Nath and Dikshith 1979; WHO 1984). Deaths occurred in humans within days after ingestion of the toxic material. In two of these cases, endosulfan was the likely primary cause of toxicity, although some synergism probably resulted from other chemicals present (e.g., alcohol, xylene). A third case involved endosulfan ingestion that resulted in death, but because another more toxic material

(dimethoate) was present in the mixture, it is difficult to attribute the toxic effects observed to endosulfan itself. In addition, the two pesticides could have acted synergistically. Absorption of endosulfan was also evidenced by the appearance of endosulfan in samples of the liver and kidney obtained from poisoning victims at autopsy (Demeter and Heyndrickx 1978; Demeter et al. 1977). In the fatal cases reported by Blanco-Coronado et al. (1992) and Lo et al. (1995) there was little doubt that death was caused by effects triggered by endosulfan. In one fatality, the concentration in the blood was 2.85 mg/L, and this patient died 8 days after ingesting endosulfan accidentally mixed with food (Blanco-Coronado et al. 1992). In 5 cases who survived and eventually recovered, the concentration of endosulfan determined in the blood on admission to the hospital ranged from 0.29 to 0.67 mg/L (Blanco-Coronado et al. 1992). α-Endosulfan, β-endosulfan, and/or endosulfan sulfate were present in the blood and urine of a 43-year-old man for at least 91 hours after he intentionally ingested approximately 260 mg/kg endosulfan, and the stomach contents contained 3,540 and 1,390 µg/kg of α-endosulfan and β-endosulfan, respectively, upon autopsy 4 days after exposure, indicating that absorption from the gut can occur over a prolonged period after a single oral exposure (Boereboom et al. 1998).

Evidence of the absorption of endosulfan following oral exposure has also been found in animal studies. In metabolic studies with ^{14}C-endosulfan, approximately 65% of the administered radioactivity was recovered from the excreta and tissues of mice 24 hours after ingesting endosulfan in their diet (Deema et al. 1966). In descending order, the highest activities per gram of organ/excreta from two mice were as follows: feces, visceral fat, urine, tissues, respired air, and blood. In an experiment that involved cannulation of the bile duct, approximately 22, 13, and 47% of a 2-mg/kg oral dose of α-endosulfan, and 15, 10, and 29% of a 2 mg/kg oral dose of β-endosulfan were collected in the feces, urine, and bile, respectively, after 48 hours. This indicates that absorption could be as high as 78 and 85% for α- and β-endosulfan, respectively. The rats eliminated 88% of the α-endosulfan (75% in feces, 13% in urine) and 87% of the β-endosulfan (68% in feces, 19% in urine) within 5 days of oral administration (Dorough et al. 1978). A single oral dose of endosulfan given to sheep was almost completely excreted in the feces (50% of the radiolabel) or in the urine (40% of the radiolabel) within 22 days after administration (Gorbach et al. 1968). These studies indicate that absorption occurs in humans and animals following ingestion of endosulfan.

2.3.1.3 Dermal Exposure

Evidence suggesting that humans absorb endosulfan through the skin was presented by Singh et al. (1992) who briefly described 22 cases of acute poisoning among subjects spraying cotton and rice fields.

The assumption of dermal absorption was based on the fact that subjects who sprayed rice fields and who suffered cuts over the legs caused by the sharp leaves of the rice plants showed the most severe toxicity. In another case report, serum endosulfan was 4 µg/L at 30 hours after an agricultural pilot was exposed dermally (and probably also by inhalation) for approximately 45 minutes in clothing that was "heavily contaminated" with endosulfan and methomyl (Cable and Doherty 1999); the dermal exposure level was not estimated, and no other measures of tissue levels of endosulfan were obtained.

Indirect evidence indicates that dermal absorption occurs in animals. Calves dusted with a 4% dust formulation of endosulfan had neurological symptoms (tremors, twitching, convulsions) and died within a day after exposure (Nicholson and Cooper 1977). Neurological effects have also been reported in preclipped rabbits and rats after repeated application of endosulfan to the skin (Dikshith et al. 1988; Gupta and Chandra 1975). Dikshith et al. (1988) reported levels of α-, β-, and total endosulfan in liver, kidney, brain, testes, fatty tissue, and blood 30 days after dermal application of endosulfan.

One animal study provided direct evidence of absorption of endosulfan following dermal exposure by quantifying the rate and extent of dermal absorption in Sprague-Dawley rats (Hoechst 1986). A single dermal application of aqueous suspensions of 0.10, 0.76, and 10.13 mg/kg ^{14}C-endosulfan to male rats resulted in binding of approximately 80% of the test material to the skin at all three dose levels, with the amount bound proportional to the amount applied (Hoechst 1986). After 10 hours, approximately 72% of the applied dose was bound to the skin, and 8% of the applied dose was absorbed into the body. After 24 hours, approximately 25% of the bound material was absorbed into the body. Absorption rates were calculated, with the highest rates occurring within the first half hour after application. For the low-, middle-, and high-dose groups, the absorption rates at 0.5 hour were 2.8, 21.7, and 453.9 µg/cm^2 of skin/hour, respectively, with the rates being proportional to the amount of endosulfan applied to the skin. The absorption rates decreased with time for all three dose groups. By 24 hours, the absorption rates were 0.1, 0.7, and 6.3 µg/cm^2 of skin/hour for the low-, middle-, and high-dose groups, respectively (Hoechst 1986).

2.3.2 Distribution

Studies in animals and autopsy findings of endosulfan and metabolites in various tissues in humans suggest that absorbed endosulfan is most readily distributed to adipose and brain tissue, but that the liver and kidney may be longer-term repositories of endosulfan and its metabolites. Endosulfan residues were

found in fat of hospitalized Spanish children (Olea et al. 1999), but no studies were located regarding known or suspected differences between children and adults with respect to endosulfan distribution.

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of endosulfan in humans and animals after inhalation exposure to endosulfan.

2.3.2.2 Oral Exposure

α-Endosulfan, β-endosulfan, and the primary metabolite, endosulfan sulfate, have been detected in several human autopsy samples following acute ingestion. In a man who had ingested endosulfan in a single oral dose of approximately 260 mg/kg, postmortem tissue concentrations of α-endosulfan at 4-days postexposure were 4,105, 80, and 59 µg/kg in the fat, brain, and kidney, respectively, the concentration of β-endosulfan in the brain was 69 µg/kg, and the concentrations of endosulfan sulfate were 3,030, 1,350, and 390 µg/kg in the liver, brain, and kidney, respectively (Boereboom et al. 1998). In 3 other suicides cases, the following concentrations for combined isomers of endosulfan were found in autopsy specimens: blood, 4–8 ppm; liver 0.8–1.4 ppm; kidney, 2.4–3.2 ppm; and brain, 0.25–0.30 ppm (Coutselinis et al. 1978). No information was available on the amount of endosulfan ingested. It is apparent that the highest concentrations were detected in the kidney, liver and blood. However, it was not possible to determine the specific levels that elicited systemic toxicity prior to death. An autopsy was performed on a 28-year-old man who ingested 20% endosulfan powder (12.4% α, 8.1% β) while drunk and was dead on arrival at the hospital. The autopsy revealed α- and β-endosulfan in the following concentrations: blood, 0.06 and 0.015 ppm; urine, 1.78 and 0.87 ppm; liver, 12.4 and 5.2 ppm; and kidney, 2.48 and 1.8 ppm; respectively. In another case, endosulfan sulfate was found in the liver at a concentration of 3.4 ppm (Demeter and Heyndrickx 1978; Demeter et al. 1977).

Endosulfan has been detected in breast milk of women environmentally exposed to a number of contaminants in rural Kazakhstan (Lutter et al. 1998), indicating that transfer to children can occur during lactation; the endosulfan concentration in the breast milk was not reported. Isomers and metabolites of endosulfan were detected in the fat of 30–40% of children hospitalized in agricultural regions of Spain, demonstrating that endosulfan accumulates in adipose tissue of children after presumably repeated dietary exposure (Olea et al. 1999). Considering evidence from a study in rats indicating that endosulfan is rapidly eliminated from fat tissues after cessation of dietary exposure (Dorough et al. 1978), it is probable

that the endosulfan residues in the fat tissues of the children do not represent long-term storage; rather, the residues probably represent recent exposures in children who were repeatedly exposed to endosulfan.

The distribution of radioactivity 24 hours after a single oral exposure to a food pellet treated with α- and β-^{14}C-endosulfan in male mice was as follows: feces > small intestine > urine > visceral fat > liver > kidney > expired carbon dioxide > blood (Deema et al. 1966). In a 14-day feeding study with 0.25 mg/kg/day of radiolabeled α- or β-endosulfan, female albino rats had the highest ^{14}C concentrations in liver and kidney (Dorough et al. 1978). The authors reported that endosulfan accumulated in fat tissues during the 14-day exposure period, then declined to undetectable levels by 7 days postexposure. Levels in the fat tissue never reached as high as those seen in the liver and kidney. Concentrations of α-endosulfan, β-endosulfan, and endosulfan sulfate in cattle fatally poisoned after a single exposure due to accidental ingestion of an unknown amount of endosulfan were reported by Braun and Lobb (1976). Total endosulfan residues (α-endosulfan, β-endosulfan, and endosulfan sulfate) were 0.083, 0.065, and 4.23 ppm in the liver; 0.04, 0.024, and 1.06 ppm in the kidney; 0.031, 0.024, and 0.61 ppm in muscle; and 720, 550, and 0.0 ppm in rumen contents, respectively. The surviving calf had 0.025 ppm endosulfan in its blood.

Rats exposed daily via gavage to 5 or 10 mg/kg/day of endosulfan in peanut oil had plasma levels of 2.26 and 0.46 µg/mL for the α- and β-isomers, respectively (Gupta 1978). These levels were measured on the day after the termination of a 15-day gavage dosing regimen. Fifteen days after the last treatment, the plasma concentration of α-endosulfan was 0.05 µg/L, while the β-isomer was not detected. Endosulfan levels were twice as high in fatty tissues as in liver and kidney following 30 days of exposure of male and female Wistar rats to endosulfan (Dikshith et al. 1984). Thirty days of exposure to 11 mg/kg/day produced the highest level in the kidney, while the fatty tissue had a slightly lower concentration (Nath and Dikshith 1979). Male rats exposed daily for 60 days to 2.5 or 3.75 mg/kg/day of endosulfan containing α- and β-isomers in a ratio of 2:1 produced somewhat different distribution patterns for the two isomers (Ansari et al. 1984). For both doses, the highest concentration of the α-isomer was detected in the kidney followed by the epididymis, ventral prostate or spleen, testes, brain, and liver. In descending order, the highest levels of the β-isomer were found in the seminal vesicle, epididymis, heart, ventral prostate, spleen, and liver. Overall, the greatest amounts of both α- and β-isomers of endosulfan were located in the kidney, seminal vesicle, and epididymis, with the liver having the least amount.

α-Endosulfan; β-endosulfan; and the endosulfan metabolites, endosulfan sulfate, endosulfan hydroxyether, endosulfan lactone, and endosulfan diol, were measured in blood, liver, and kidney from

male rats consuming 34 or 68 mg/kg/day of endosulfan over 4 weeks and from male rats given a 30-day recovery from exposure (Hoechst 1987). Only trace amounts of endosulfan and its metabolites were found in the blood. The predominant substances found in the liver were endosulfan sulfate and endosulfan lactone. Trace amounts of α- and β-endosulfan were measured in the liver; however, there was substantial accumulation of α-endosulfan in the kidneys. Approximately 200 times more α-endosulfan than β-endosulfan was found in the kidney. The predominant metabolites of endosulfan found in the kidney were endosulfan sulfate and endosulfan-lactone. Endosulfan-diol was also found, but at much lower concentrations. The amount of endosulfan found in the kidney decreased following a period free from exposure. By the end of the recovery period (4 weeks), α- and β-endosulfan and the endosulfan metabolites were reduced to trace levels in all organs (Hoechst 1987).

Information regarding transfer of endosulfan residues to offspring through breast milk is available from a study in lactating goats (Indraningsih et al. 1993). Goats were administered a daily dose of 1 mg/kg for 28 days and adults and kids were sacrificed at various times (days 1, 8, 15, 21 posttreatment) after treatment ceased. With the exception of the kidneys, the highest concentration of residues were recorded in the adults on day 1. In the kidneys, residues increased from day 1 to a maximum on day 8. On day 1, the concentration of residues in the kidneys, liver, and milk were 0.29, 0.20, and 0.02 mg/kg, respectively. No residues could be detected in milk on day 8. α-Endosulfan was the major residue in all tissues except for liver and fat which contained mainly endosulfan sulfate. No endosulfan residues were detected in the tissues of kids except for α-endosulfan in the liver at a concentration of 0.0011 mg/kg on day 1. These results suggest rapid elimination of residues from tissues and limited transfer to offspring through breast milk. Approximately 1% of the radiolabel from administration of a single oral dose of ^{14}C-endosulfan (65% α-isomer, 35% β-isomer) to milk sheep was recovered in the milk, primarily as endosulfan sulfate (Gorbach et al. 1968).

The distribution in animals after exposure to endosulfan indicates that the α-isomer of endosulfan accumulates throughout the body to a greater extent than the β-isomer (Ansari et al. 1984; Hoechst 1987). Endosulfan is distributed to the fatty tissues initially after exposure, while a greater accumulation of endosulfan reaches the kidney following prolonged exposure. Of all the metabolites of endosulfan, endosulfan sulfate appears to be the one that accumulates predominantly in the liver and kidneys (Hoechst 1987).

2.3.2.3 Dermal Exposure

Serum endosulfan was 4 µg/L at 30 hours after an agricultural pilot was exposed dermally (and probably also by inhalation) for approximately 45 minutes in clothing that was "heavily contaminated" with endosulfan and methomyl (Cable and Doherty 1999); the dermal exposure level was not estimated and no other measures of tissue levels of endosulfan were obtained. A study by Kazen et al. (1974) has identified endosulfan residues on the hands of workers after relatively long periods free from exposure. Endosulfan residues were identified on the hands of one worker approximately 30 days after exposure and on the hands of one worker who had not used endosulfan during the preceding season.

Three animal studies were located regarding distribution of endosulfan in animals following dermal exposure (Dikshith et al. 1988; Hoechst 1986; Nicholson and Cooper 1977). Endosulfan was detected in the brain (0.73 ppm), liver (3.78 ppm), and rumen contents (0.10 ppm) of calves that died after dermal exposure to a dust formulation of endosulfan (Nicholson and Cooper 1977). Following a single dermal application of aqueous suspensions of 0.1, 0.83, and 10.13 mg/kg ^{14}C-endosulfan to male Sprague-Dawley rats, low concentrations of endosulfan (ng/g levels) appeared in the blood and tissues (other than skin at and around the application site) after 1 hour (Hoechst 1986). The concentrations of endosulfan in the blood and tissues increased with the time of exposure and were proportional to the dose applied. The liver and kidney appeared to sequester radiolabel relative to the concentrations of radiolabel in the blood or fat. Endosulfan levels were approximately 10 times higher in the liver and kidney than in the fat, blood, and brain throughout the study (Hoechst 1986).

Tissue disposition of the α- and β-isomers has also been quantified in rats after intermediate-duration dermal application of technical-grade endosulfan for 30 days (Dikshith et al. 1988). Male rats were exposed to 18.8, 37.5, or 62.5 mg/kg/day; females were exposed to 9.8, 19.7, or 32.0 mg/kg/day. Fatty tissue contained the highest levels of both α- and β-isomers in both males and females. The levels (in ppb) of the α- and β-isomers, respectively, in animals exposed to the lowest doses was as follows: fatty tissue (0.26, 0.15) > kidney (0.16, 0.01) > blood (0.03, 0.015) > liver (0.02, 0.004) > brain (0.03, not detected) in male rats, and fatty tissue (2.4, 5.8) > liver (0.50, 1.2) > blood (0.56, 1.2) > kidney (0.65, 0.52) > brain (0.21, not detected) in female rats. Generally, these values increased with increased dose. The residue level of both isomers in fatty tissue was much higher in females (8.20–16.13 ng/g) than in males (0.42–0.62 ng/g).

2.3.2.4 Other Routes of Exposure

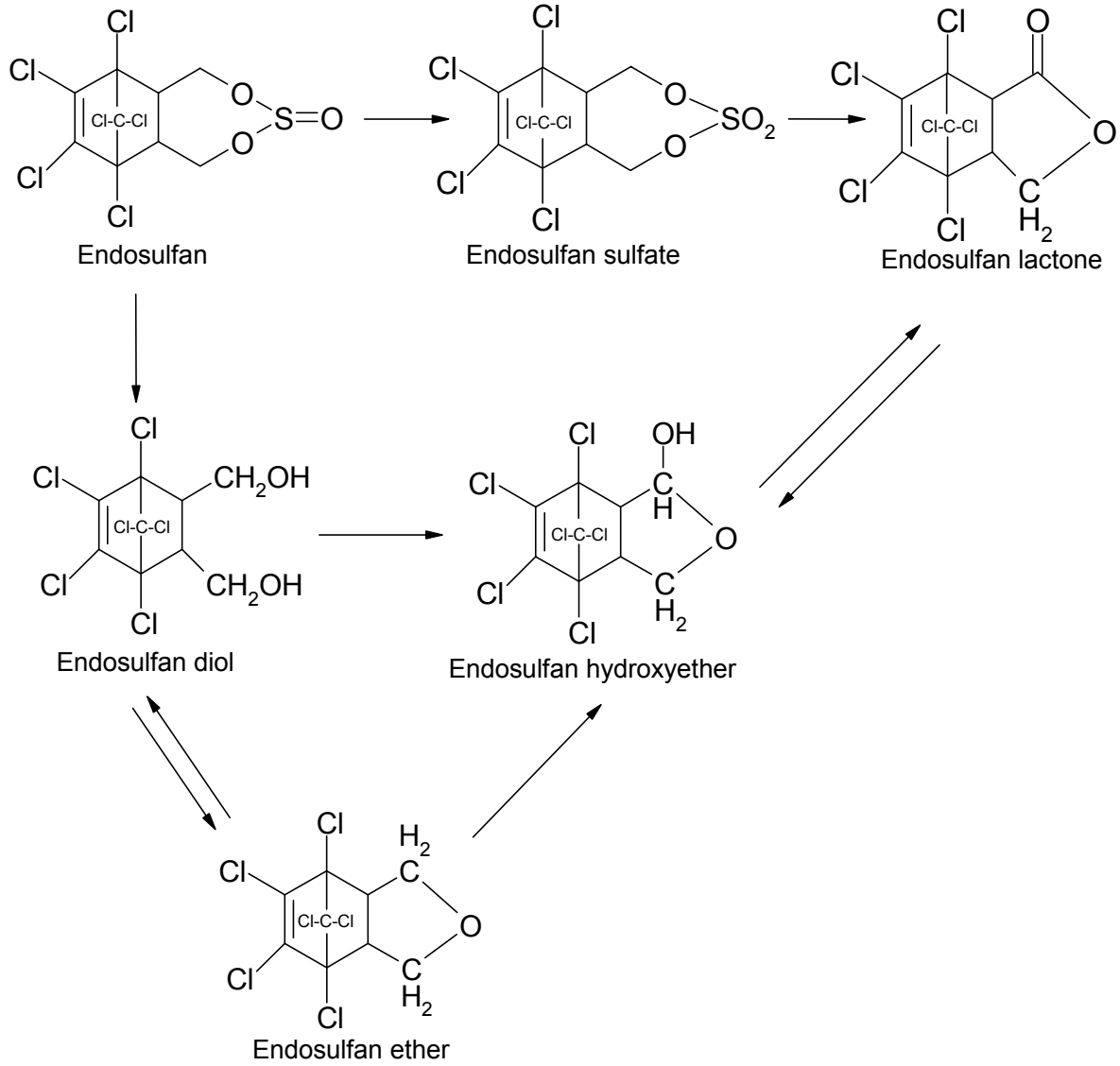
The distribution of endosulfan and endosulfan sulfate was evaluated in the brains of cats given a single intravenous injection of 3 mg/kg endosulfan (Khanna et al. 1979). Peak concentrations of endosulfan in the brain were found at the earliest time point examined (15 minutes after administration) and then decreased. When tissue levels were expressed per gram of tissue, little differential was observed in distribution among the brain areas studied. However, if endosulfan levels were expressed per gram of tissue lipid, higher initial levels were observed in the cerebral cortex and cerebellum than in the spinal cord and brainstem. Loss of endosulfan was most rapid from those areas low in lipid. Endosulfan sulfate levels peaked in the brain at 1 hour postadministration. In contrast, endosulfan sulfate levels in liver peaked within 15 minutes postadministration. The time course of neurotoxic effects observed in the animals in this study corresponded most closely with endosulfan levels in the central nervous system tissues examined.

2.3.3 Metabolism

No information is available on the metabolism of endosulfan in adult humans or children. Endosulfan is readily metabolized in animals following exposure (Deema et al. 1966; Dorough et al. 1978; Gorbach et al. 1968). It exists in two stable stereoisomeric forms, which can be converted to endosulfan sulfate and endosulfan diol (WHO 1984). These can be further metabolized to endosulfan lactone, hydroxyether, and ether. Figure 2-3 shows the pathway for the degradation of endosulfan. Dorough et al. (1978) indicated that the major portion of residues in the excreta and/or tissues consisted of unidentified polar metabolites that could not be extracted from the substrate, whereas the nonpolar metabolites, including sulfate, diol, α-hydroxyether, lactone, and ether derivatives of endosulfan, represented only minor amounts. Excretion data from an acute dermal study in rats showed that, after 24 hours, a dose-related decrease in excretion occurred at higher doses, suggesting saturation of the metabolic pathway of endosulfan (see Section 2.3.4.3) (Hoechst 1986).

High concentrations of endosulfan sulfate were found primarily in the liver, intestine, and visceral fat 24 hours after mice were exposed to a single dose of ^{14}C-endosulfan (Deema et al. 1966). Five days following a single oral administration of ^{14}C-endosulfan to rats, the diol, sulfate, lactone, and ether metabolites were detected in the feces (Dorough et al. 1978). In sheep, endosulfan sulfate was detected in the feces, and endosulfan alcohol and α-hydroxyether were detected in the urine (Gorbach et al. 1968).

Figure 2-3. Proposed Metabolic Pathway for Endosulfan



Adapted from:

Dorough et al. (1978)
Gorbach et al. (1968)

All the metabolism studies indicate that the parent compound was also found to a large degree in the tissues and excreta. Similar conclusions can be drawn from the work of Gupta and Ehrnebo (1979) who found that almost half of the parent compound was excreted unchanged in rabbits after endosulfan was injected intravenously. The metabolites (e.g., endosulfan sulfate, endosulfan diol) were reported in tissues and excreta following longer exposures to endosulfan (Deema et al. 1966; Dorough et al. 1978). Based on the rapid appearance of endosulfan sulfate in the liver following intravenous administration of endosulfan, it may be concluded that the liver is a site of high metabolic activity in the conversion of endosulfan to endosulfan sulfate (Khanna et al. 1979).

Results of a study in which male rats were fed 34 or 68 mg/kg/day endosulfan over 30 days suggest that metabolism of endosulfan occurs in the kidney (Hoechst 1987). This feeding study was initiated to clarify findings from a previous 13-week feeding study with endosulfan in which a yellow discoloration was observed in the kidneys of rats fed diets containing up to 360 ppm. The results of the 30-day feeding study showed that endosulfan accumulates predominantly in the kidney during exposure and that storage of endosulfan in the kidney is reversible upon removal from exposure (Hoechst 1987). Histological examination of the kidney revealed granular pigmentation and an increase in the number and size of lysosomes in the cells of the proximal convoluted tubules in the kidneys (Hoechst 1987). These changes diminished appreciably during the 30-day recovery period. These lysosomal changes may suggest storage or metabolism of endosulfan in the kidneys (Hoechst 1987). The lysosomal sequestration of endosulfan may account for the yellow pigment seen in the kidneys in a previous toxicity study. The diminishing pigmentation and decreasing endosulfan concentrations, which occurred during the 30-day recovery period, suggest metabolism of the compound in the kidney (Hoechst 1987).

Evidence suggests that endosulfan can induce microsomal enzyme activity. Increased liver microsomal cytochrome P-450 activity was observed in male and female rats after single and multiple administrations of endosulfan (Siddiqui et al. 1987a; Tyagi et al. 1984). Increased enzyme activity was observed in hepatic and extrahepatic tissues. Based on the increase in aminopyrine-*N*-demethylase and aniline hydroxylase activity, endosulfan has been shown to be a nonspecific inducer of drug metabolism (Agarwal et al. 1978).

The available evidence indicates that endosulfan can be metabolized in animals to other lipophilic compounds, which can rapidly enter tissues, and to more hydrophilic compounds that can be excreted.

2.3.4 Elimination and Excretion

Renal excretion is the most important endosulfan elimination route in humans and animals. Biliary excretion has also been demonstrated to be important in animals. Estimated elimination half-lives ranged between approximately 1 and 7 days in adult humans and animals. Endosulfan can also be eliminated via the breast milk in lactating women and animals, although this is probably a relatively minor elimination route. No studies were located regarding known or suspected differences between children and adults with respect to endosulfan excretion.

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in animals after inhalation exposure to endosulfan.

The concentration of α- and β-endosulfan in the urine of a pest control worker who wore protective equipment peaked at 0.2 days (approximately 5 hours) after completing a 25-minute application of endosulfan in a greenhouse, declined to control levels by about 1.5 days postexposure, and remained at levels comparable to controls until the end of sampling at 3-days postexposure (Arrebola et al. 1999). Assuming first-order elimination, the urinary elimination half-life was estimated to be 0.94 days for α-endosulfan and 1.16 days for β-endosulfan; no endosulfan metabolite was detected in any urine sample.

2.3.4.2 Oral Exposure

α-Endosulfan and endosulfan sulfate, but not β-endosulfan, were observed in the urine at 0–3.5 hours after exposure, and endosulfan sulfate was also observed in the urine up to 91 hours after exposure in a man who died from ingesting endosulfan in a single oral dose of approximately 260 mg/kg (Boereboom et al. 1998); the terminal half-lives of α- and β-endosulfan in a two-compartment toxicokinetic model were 24.3 and 60.4 hours, respectively. In another case report, endosulfan was quantified in the urine of four patients who ingested endosulfan several hours earlier (Blanco-Coronado et al. 1992). Since the amount ingested was not known, the percentage of the ingested dose that was excreted could not be determined. It is also unknown whether the urine was the main route of excretion.

Endosulfan residues are rapidly eliminated from tissues as suggested by a half-life of approximately 7 days estimated in a 14-day oral study in female rats (Dorough et al. 1978). Rapid elimination was also

observed in a 28-day study in goats in which half-lives between 1.1 and 3.1 days were estimated for endosulfan residues in various organs and tissues (Indraningsih et al. 1993).

Orally administered endosulfan is eliminated in both the feces and the urine of mice and rats, with the feces containing most of the pesticide eliminated (Deema et al. 1966; Dorough et al. 1978). In a study using rats, endosulfan was orally administered as a single gavage dose (2 mg/kg) or in the diet (0.25 or 1.25 mg/kg/day) for 2 weeks (Dorough et al. 1978). The animals given the oral single dose eliminated 19 and 25% of the α- and β-isomer dose in the feces and the urine, respectively, in the form of the original compound and its metabolites 24 hours after exposure. After 120 hours, the percentages increased to 88 and 87%, respectively. The cumulative ratio of α-endosulfan eliminated in the feces compared with the urine after 120 hours was 5:1. The ratio for β-endosulfan was somewhat less than 7:2. These ratios are equivalent to excretion of 17% α-endosulfan and less than 22% β-endosulfan in the urine. The authors found that collection of bile from the rats caused a decrease in the elimination of endosulfan and its metabolites in the feces but had no effect on urinary excretion. Assuming little enterohepatic recirculation, data from bile duct cannulation indicate that about 65–70% of the amount in the feces was due to biliary excretion. Animals administered endosulfan in the diet for 2 weeks showed a similar elimination pattern. The total cumulative percentage of the radiocarbon eliminated from rats given the α- or β-isomers in the diet (5 ppm) was 64 and 65%, respectively. Ratios of the cumulative dose eliminated via the feces compared with the urine for both isomers were approximately 7:1. No major differences were noted in animals given 25 ppm endosulfan in the diet. Extraction and analysis of the feces showed that the residues consisted of the parent compound, five polar metabolites, and unidentified polar material. The urine and feces contained the diol, α-hydroxyether, and lactone of endosulfan. Furthermore, these authors indicated that 47% of the dose was eliminated from the liver via biliary secretion 48 hours following treatment. In mice, endosulfan sulfate and alcohol (diol) were the main metabolites detected, primarily in the feces (Deema et al. 1966). The ratio of radioactivity recovered per gram of excreta for feces and urine was 26:1; however, no corrections were made for quenching or self-absorption. Administration of a single oral dose of ^{14}C-endosulfan (65% α-isomer, 35% β-isomer) to milk sheep resulted in recovery of approximately 50% of the radiolabel in the feces, 4% in the urine, and 1% in the milk (Gorbach et al. 1968). Unmetabolized endosulfan was found in the feces but not in the urine. The main metabolites found in the urine were endosulfan diol and α-hydroxyendosulfan ether. Most of the ^{14}C activity in the milk of sheep was due to endosulfan sulfate. Although these studies suggest variations in the excretion patterns with different species, they do provide evidence that the excretion of endosulfan and its metabolites after oral exposure is rapid and occurs mainly through the fecal route.

Gavage dosing of male and female rats with endosulfan (65.3% α-endosulfan, 33.7% β-endosulfan) for 30 days resulted in a greater accumulation of endosulfan in fatty tissue from females than males (Dikshith et al. 1984). The authors speculated that the difference between males and females was a function of more rapid excretion of endosulfan by males than females, and that this could account for the higher sensitivity of female rats to endosulfan toxicity. However, excretion of endosulfan and its metabolites was not directly measured in this study; therefore, alternative explanations for the differences in residue content and toxicity cannot be discounted.

2.3.4.3 Dermal Exposure

Endosulfan and metabolites were observed in the urine of workers who had prepared and applied endosulfan for 2–5 hours either 1 day or 1 week prior to sampling, without using protective clothing or face mask (thus, exposure was probably both dermal and inhalation) (Vidal et al. 1998). Unchanged α- and β-endosulfan and endosulfan ether were the predominant chemicals excreted 1 day following exposure. One week after exposure, α-endosulfan was detected in urine of four of five workers, but β-endosulfan was detected in only one of five samples and endosulfan ether was not detected at all. Endosulfan sulfate was detected in only one of five samples at 1 week after exposure and in none of the four samples at 1 day postexposure. Endosulfan lactone was detected in one of four and one of five samples at 1 day and 1 week after exposure, respectively.

One study was located regarding excretion in animals after dermal exposure to endosulfan (Hoechst 1986). Following a single dermal application of an aqueous suspension of ^{14}C-endosulfan (at 0.1, 0.83, 10.13 mg/kg) to male Sprague-Dawley rats, limited excretion of radiolabel (0.5–1.0% of the applied dose) occurred during the first 10 hours of exposure and occurred primarily in the urine. However, between 10 and 24 hours, excretion increased to an average of 10% of the absorbed dose. Elimination was rapid once the endosulfan passed through the skin. Excretion was dose related (13.5% of the absorbed dose at the low dose; 12.4% at the middle dose; and 4.9% at the high dose), with the percentage excreted decreasing with increasing dose. Although excretion was greater in the urine than feces during the first 10 hours of exposure, by 24 hours, excretion in the feces was approximately two times greater than in the urine (Hoechst 1986).

2.3.4.4 Other Routes of Exposure

Intravenous administration of endosulfan (7:3 ratio of α- and β-isomers) in rabbits produced slower elimination of the α-isomer (Gupta and Ehrnebo 1979). Excretion of the two isomers occurred primarily via the urine (29%) with much less excreted via the feces (2%). Given the earlier evidence in rats and mice describing the principal route of elimination of endosulfan and its metabolite to be via the feces, the differences in the excretion pattern in this study may be attributable to differences in exposure routes, to species differences, or to both. Nevertheless, studies in laboratory animals suggest that both renal and hepatic excretory routes are important in eliminating endosulfan from the body. Elimination of small doses is essentially complete within a few days.

2.3.5 Physiologically based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of

toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

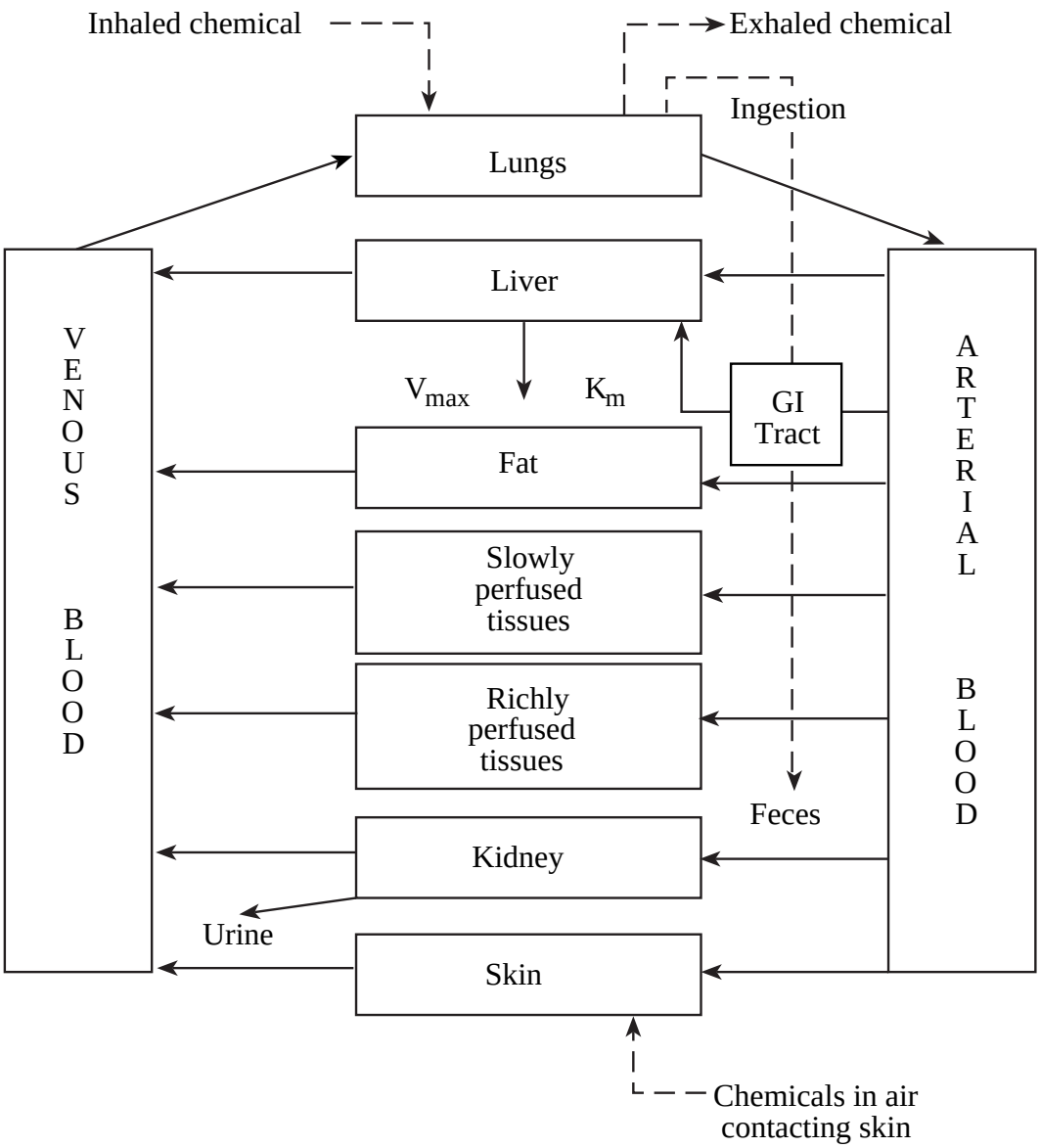
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

If PBPK models for endosulfan exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

No PBPK modeling studies were located for endosulfan.

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

No information was located regarding the mechanism of inhalation, oral, or dermal absorption of endosulfan in humans or animals; however, the lipophilic nature of endosulfan suggests that it is probably absorbed by passive diffusion. Also, no information was located regarding the mechanism by which endosulfan is transported in the blood. However, due to endosulfan's high solubility in lipids, it is reasonable to assume that in the blood it might be associated with a lipid fraction. Studies in animals suggest that endosulfan initially accumulates in fatty tissues and that relatively high amounts can be found in the liver and kidneys after exposure (Dorough et al. 1978; Gupta 1978; Hoechst 1987; Nath and Dikshith 1979). Rapid accumulation of endosulfan metabolites in the liver (Khanna et al. 1979) and increased lysozymal activity in the kidney (Hoechst 1987) suggest that these may be sites of endosulfan metabolism. Although endosulfan induces microsomal cytochrome P-450 in the liver (Siddiqui et al. 1987a; Tyagi et al. 1984), it is not clear whether endosulfan thereby induces its own metabolism. Results from a dermal study in rats suggested that the metabolism of endosulfan may be a saturable process (Hoechst 1986). In animals, biliary excretion of endosulfan and metabolites is a main route of elimination of this chemical and may contribute about two-thirds of the endosulfan found in the feces (Dorough et al. 1978). A minor proportion of endosulfan and metabolites is excreted in the urine.

2.4.2 Mechanisms of Toxicity

The neurotoxic effects of endosulfan are well documented in both humans and animals, and extensive research has been conducted in recent years aimed at elucidating its mechanism of neurotoxicity. Although serious neurotoxic effects, including death, generally occur after acute exposure to concentrations much higher than those commonly found in the environment, there is concern about the possibility of accidental exposure of those occupationally exposed such as agricultural workers who apply the pesticide in the fields. In addition to neurotoxicity, exposure to endosulfan in animals has induced a wide array of effects including liver and kidney toxicity, hematological effects, alterations in the immune system, and alterations in the reproductive organs of males. The possible mechanisms of the effects on organ or systems other than the nervous system have not been as well studied as the mechanism of neurotoxicity. Speculation in this section on the mechanism(s) of action involved in effects that have not been well characterized and/or have been seen inconsistently in animal studies seems inappropriate at this time. Yet, where possible explanations for some effects have been suggested by the investigators

conducting the research, these are presented in the appropriate subsections in Section 2.5. Therefore, this section will focus mainly on the mechanism of neurotoxic effects of endosulfan.

Acute exposure to large amounts of endosulfan results in frank effects manifested as hyperactivity, muscle tremors, ataxia, and convulsions. Possible mechanisms of toxicity include (a) alteration of neurotransmitter levels in brain areas by affecting synthesis, degradation, and/or rates of release and re-uptake, and/or (b) interference with the binding of those neurotransmitter to their receptors.

Several studies have reported changes in neurotransmitter levels following exposure to endosulfan. For example, Gupta (1976) found that brain acetylcholinesterase activity was decreased following a single intraperitoneal injection of endosulfan in rats and postulated that the decreased activity of this enzyme resulted in an increase in brain levels of acetylcholine, which could, in turn, be responsible for the central nervous system stimulation observed. However, brain cholinesterase was increased in female rats that consumed 4.59 mg/kg/day and above for 13 weeks (Hoechst 1985a). Thus, it is unclear whether the decrease in brain acetylcholinesterase observed by Gupta (1976) was a representative finding. Neither Paul et al. (1994) nor Lakshmana and Raju (1994) found changes in the activity of acetylcholinesterase in the brain of rats treated with 2 mg endosulfan/kg/day for 90 days or with 6 mg/kg/day for 23 days, respectively. Ansari et al. (1987) also suggested that changes in neurotransmitter levels (specifically serotonin, GABA, and dopamine) in the brain may be partly responsible for the neurotoxicity of endosulfan in rats after observing hyperactivity, tremors, and convulsions following a single intra-peritoneal injection of 40 mg/kg of endosulfan. More recently Paul et al. (1994) found significant increases in serotonin concentration in the cerebrum and midbrain of rats after 90 days of treatment with 2 mg/kg/day endosulfan, and in this study, spontaneous motor activity was significantly increased in the treated animals. Furthermore, Paul et al. (1994) also found a correlation between the increase in serotonin and inhibition of a learning paradigm. Lakshmana and Raju (1994) also reported changes in the concentrations of dopamine, noradrenaline, and serotonin in various brain areas of endosulfan-treated rats. In this case, treated rats took 29% more time to learn a behavioral task; however, it was not determined which neurotransmitter(s) change may have been responsible for the behavioral change.

Studies have also examined the role of neurotransmitter receptors in endosulfan-induced neurotoxic effects. For instance, a single intraperitoneal dose of 3 mg/kg of endosulfan or administration of 1 mg/kg/day for 30-days had no effect on frontal cortical ^{3}H-serotonin binding or aggressive behavior in adult rats, but 30 daily injections of 3 mg/kg/day caused a significant increase in ^{3}H-serotonin binding affinity and foot-shock-induced fighting (Agrawal et al. 1983). Serotonin may also play a role in the

increase in aggressive behavior (foot-shock-induced fighting) observed in rats following multiple exposures to endosulfan (Agrawal et al. 1983; Zaidi et al. 1985). Rat pups injected with 1 mg/kg/day for 25 days showed a significant increase in frontal cortical ^{3}H-serotonin binding and exhibited a significant increase in foot-shock-induced fighting behavior (Zaidi et al. 1985). These effects were still observed 8 days after cessation of treatment. The authors concluded that endosulfan affects serotonergic function, which in turn induces neurotoxicity in both neonates and adults, as demonstrated by increased ^{3}H-serotonin binding to the frontal cortex and aggressive behavior. A correlation between ^{3}H-serotonin binding and aggressive behavior was also observed. These data also suggest that neonates show a greater sensitivity to endosulfan than adults.

The results from several studies suggest the involvement of GABA receptors in endosulfan-induced neurotoxicity. In a series of *in vitro* experiments using ^{3}H-dihydropicrotoxinin, Abalis et al. (1986), Cole and Casida (1986), Gant et al. (1987), and Ozoe and Matsumura (1986) showed that endosulfan acts as a noncompetitive GABA antagonist at the chloride channel within the GABA receptor in brain synaptosomes. Antagonism of GABAergic neurons within the central nervous system leads to generalized central nervous system stimulation. Binding of GABA to its receptor opens chloride-selective ion channels leading to influx of chloride into neurons through electrochemical gradient, resulting in hyperpolarization of the membrane and inhibition of cell firing. A reduced inhibitory drive translates into increased activity of the effector neurons. The studies mentioned above found that the ability of endosulfan to induce convulsions correlated with the potency to bind to this site and to inhibit GABA-induced chloride flux, thus providing good evidence for this mechanism of action. A more recent study showed that α-endosulfan blocked the chloride uptake induced by GABA in primary cultures of cortical neurones from 15-day old mice fetuses by interacting with the t-butylbicyclophosphorothionate (a GABA antagonist) binding site (Pomes et al. 1994). In a subsequent study, the same group of investigators found that α-endosulfan had relatively low cytotoxicity (assessed by disruption of cell membrane integrity) in primary neuronal cultures of cerebellar granule cells, and that it did not increase the formation of intracellular oxidative radicals (Rosa et al. 1996). It did, however, increased mitochondrial transmembrane potential which, according to Rosa et al. (1996), could be linked to a detoxification process of the cell. The authors further stated that their findings were consistent with the view that *in vivo* neurotoxicity is mediated mainly by inhibition of GABAergic function and that other effects detected *in vitro* are less important. Currently, the GABA-antagonism mechanism of toxicity is the most widely accepted hypothesis.

As summarized in Section 2.3.3 (Metabolism), the biotransformation of endosulfan can give rise to a number of both polar and nonpolar metabolites. There is little and inconclusive information on whether the toxicological properties of endosulfan are due to the parent compound or to any of its metabolites. One could assume that the more lipophilic substances will cross cell membranes more easily than polar metabolites, accumulate to a greater extent, and perhaps be the most neurotoxic. Differential toxicity could also be related to differential affinity for the GABA receptor. What is known from oral acute-lethality studies in rats and mice is that α-endosulfan is approximately 3 times more toxic than β-endosulfan (Dorough et al. 1978; Hoechst 1975, 1990; Maier-Bode 1968). In addition, in mice, the acute toxicity of endosulfan sulfate was comparable to that of α-endosulfan (Dorough et al. 1978). Also in mice, the metabolites endosulfan α-hydroxy ether, endosulfan lactone, and endosulfan ether had lethal doses 10–20 times higher than the α-or β-isomers; the lethal dose for endosulfandiol was two orders of magnitude higher than that of the α-or β-isomer (Dorough et al. 1978). Extrapolation of this information to possible potency differences in longer-term studies is clearly inappropriate since other factors, such as pharmacokinetics and possibly induction of biotransformation enzymes, play a role in longer-term studies.

Evidence from some oral studies in rats suggests that there is a difference in susceptibility to some effects of endosulfan between males and females. For example, the LD_{50} values in females were 3–4 times lower than in males (Hoechst 1990), and similar observations had been made by others (Gupta 1976; Gupta and Chandra 1977). Also, in a 30-day feeding study, 3 out 10 females, but no males, died during the study and the female survivors experienced more pronounced liver toxicity than the males (Paul et al. 1995). The higher sensitivity of females is thought to be due to a greater accumulation and slower elimination of endosulfan residues than the males (Dikshith et al. 1984, 1988). Paul et al. (1995) also conducted a series of motor and neurobehavioral tests in both sexes and found that although endosulfan increased spontaneous motor activity in both sexes, the increase was significantly greater in males. They speculated that males may produce more lipophilic metabolites, such as endosulfan sulfate, than females, which could be responsible for the more marked stimulation of spontaneous activity in males. If this were the case, then endosulfan residues other than the sulfate would be responsible for the adverse liver effects. No consistent differential sensitivity has been observed in species other than the rat.

Human data as well as studies in animals have provided negative evidence of carcinogenicity for endosulfan (Hack et al. 1995; Hoechst 1988b, 1989a). However, endosulfan promoted the development of altered hepatic foci in rats initiated with nitrosodiethylamine (Fransson-Steen et al. 1992). Although the mechanism of tumor promotion of endosulfan is not known, it has been suggested that it involves

inhibition of cellular communication (Kenne et al. 1994). A brief discussion of this topic is provided in Section 2.5 under Cancer Effects.

2.4.3 Animal-to-Human Extrapolations

Almost all the information regarding the effects of endosulfan in humans is derived from cases of acute exposure to high amounts of the chemical. Some of these cases resulted in death (Bernardelli and Gennari 1987; Blanco-Coronado et al. 1992; Demeter and Heyndrickx 1978; Lo et al. 1995; Terziev et al. 1974). Postmortem examination revealed lesions to a variety of organs and tissues, and this is consistent with findings in animals exposed to lethal doses of endosulfan. In both humans and animals, high doses of endosulfan affect primarily the nervous system. However, whether effects seen in animals exposed to lower doses of endosulfan for prolonged periods of time would also manifest in humans under similar exposure conditions remains to be determined. Also, there is not enough information to predict whether the metabolism and disposition of endosulfan by humans is similar to those in experimental animals.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview. Humans living in areas surrounding hazardous waste sites may be exposed to endosulfan primarily via dermal contact with or ingestion of contaminated soils since this compound is found bound to soil particles. Although endosulfan can be found in water as colloidal suspensions adsorbed to particles, ingestion of contaminated finished drinking water is not expected to be a major route of exposure since endosulfan is not very water soluble. Likewise, inhalation exposure to endosulfan via volatilization from contaminated media is not a major route of exposure since endosulfan is not very volatile. For the general population (including individuals not living in the vicinity of hazardous waste sites), the most likely route of exposure to endosulfan is via ingestion of residues on contaminated food. Issues relevant to children are explicitly discussed in 2.7 Children's Susceptibility and 5.6 Exposures of Children.

The clinical signs common to both humans and animals after acute exposure to high doses of endosulfan (e.g., hyperactivity, tremors, decreased respiration, dyspnea, salivation, tonic-clonic convulsions, and death) point to the nervous system as the major target of toxicity. However, neurotoxic effects are generally not seen following longer-term, low-dose exposure. Information regarding effects of endosulfan in humans is derived mainly from studies of occupational exposure and cases of intentional or accidental ingestion of endosulfan. The available occupational studies have limitations, including lack of

precise exposure data and presence of other compounds, as well as confounding factors. No information was located regarding immunological, reproductive, or developmental effects in humans exposed to endosulfan. No reports of cancer in humans associated with exposure to endosulfan were found. Endosulfan has not caused cancer in animals under the experimental conditions tested, but some found evidence of promotion activity. Target organs of endosulfan identified in experimental animals but not humans include the gastrointestinal tract, blood, liver, kidney, reproductive organs, and immune system. Developmental toxicity has also been noted in animals. There is conflicting evidence from animal studies as to whether young animals are more susceptible to the effects of endosulfan than older animals. Effects observed on the respiratory and cardiovascular systems are most likely secondary to effects of endosulfan on the central nervous system control of respiratory and cardiovascular function. Very few studies have examined the toxicity of endosulfan following inhalation or dermal exposure in humans or animals, but the effects reported (e.g., central nervous system stimulation and hepatic and renal effects) are similar to those seen after oral exposure. There is no evidence indicating that the effects of endosulfan are route-specific. A major metabolite of endosulfan, endosulfan sulfate, which is also found at some hazardous waste sites, is reported to have similar toxicity (WHO 1984).

Minimal Risk Levels for Endosulfan.

Inhalation MRLs.

Information regarding inhalation exposure to endosulfan by humans was inadequate for derivation of inhalation MRLs (Aleksandrowicz 1979; Ely et al. 1967). Limitations associated with these reports include lack of quantitative exposure data, lack of data on the duration of exposure, the possibility of multiple routes of exposure (i.e., oral and dermal, as well as inhalation), and possible concurrent exposure to other chemicals. Therefore, this information can only provide qualitative evidence of adverse effects associated with inhalation exposure to endosulfan in humans. No acute inhalation MRL was derived for endosulfan based on animal data because the most sensitive effects observed after acute inhalation exposure were trembling and ataxia in rats exposed to 3.6 mg/m^3 for 4 hours (Hoechst 1983a). These effects are considered by ATSDR to be serious effects, and MRLs are not derived using NOAELs or LOAELs for serious end points. No intermediate-duration inhalation MRL was derived because of a lack of suitable NOAELs or LOAELs. A study showed no observable systemic, immunologic, neurologic, or reproductive toxicity following inhalation exposure of rats to 2 mg/m^3 for 6 hours/day, 5 days/week for a total of 21 out of 29 days (Hoechst 1984c). However, in the absence of a body of data showing a continuum of effects at a range of doses, the use of a free-standing NOAEL for derivation of an MRL

value is not recommended. No chronic-duration inhalation MRL was derived because of a lack of studies examining the effects of chronic-duration inhalation exposures.

Oral MRLs.

No acute-duration oral MRL was derived for endosulfan because no suitable end point was available among the reliable acute-duration studies. The lowest LOAEL, 1.8 mg/kg/day, was for a serious end point, convulsions in pregnant rabbits, appearing 10 days after the start of daily gavage dosing in the FMC (1981) study. Because animals from both the control and the test groups developed ascites, and six rabbits were added without concurrent controls, the reliability of these results is questionable.

- An MRL of 0.005 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to endosulfan.

The intermediate-duration oral MRL was derived based on the observation of decreases in humoral and cell-mediated immune responses in rats consuming 0.9 mg/kg/day for 22 weeks (Banerjee and Hussain 1986). Choice of this end point is supported by the observation of similar effects in rats at higher doses following ingestion for shorter periods (Banerjee and Hussain 1986, 1987) and decreased neutrophils and spleen weight at slightly higher doses in mice (Hoechst 1984b). The absence of observed immunotoxicity in the study by Vos et al. (1982) does not contradict these findings since not all of the same end points were evaluated in the study by Vos et al. (1982), and a shorter period of exposure was used. In support of the positive findings, Khurana et al. (1998) observed decreased macrophage functionality, in the absence of any other apparent toxicological effects, in 1-day-old broiler chicks fed 30 ppm endosulfan in the diet for 4 or 8 weeks. The intermediate-duration MRL of 0.005 mg/kg/day was derived by dividing the NOAEL for immunotoxicity (0.45 mg/kg/day) by an uncertainty factor of 100 (10 for extrapolating from animals to humans and 10 for human variability).

- An MRL of 0.002 mg/kg/day has been derived for chronic-duration (365 days or more) oral exposure to endosulfan.

The chronic-duration oral MRL was derived based on the observation of increased serum levels of alkaline phosphatase (an indicator of hepatotoxicity) in dogs consuming 0.6 mg/kg/day for 1 year (Hoechst 1989c). The choice of this end point is supported by the observation of hydropic hepatic cells in rats that consumed 5 mg/kg/day for 2 years (FMC 1959b). The chronic-duration MRL of 0.002 mg/kg/day was derived by dividing the NOAEL for elevated serum alkaline phosphatase

(0.18 mg/kg/day) by an uncertainty factor of 100 (10 for extrapolating from animals to humans, and 10 for human variability).

No acute-, intermediate-, or chronic-duration dermal MRLs were derived for endosulfan because of the lack of an appropriate methodology for the development of dermal MRLs.

Death. Endosulfan has been fatal to humans following accidental and intentional ingestion of concentrated endosulfan solutions (Bernardelli and Gennari 1987; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Demeter and Heyndrickx 1978; Lo et al. 1995; Terziev et al. 1974). Death has also been observed in animals following inhalation (Hoechst 1983a), oral (Boyd and Dobos 1969; Boyd et al. 1970; Chatterjee et al. 1986; FMC 1958, 1980a, 1980b; Gupta et al. 1978, 1981; Hoechst 1966a, 1966b, 1970, 1975, 1988a), and dermal (Gupta and Chandra 1975; Hoechst 1989b; Nicholson and Cooper 1977) exposure to endosulfan, but no such cases have been reported in humans following inhalation or dermal exposures. Death in humans and animals is generally attributed to respiratory arrest following convulsive seizures.

According to Blanco-Coronado et al. (1992), acute intoxication with endosulfan involves two stages: gastrointestinal symptoms, tonic-clonic convulsions, respiratory depression, metabolic acidosis, and hyperglycemia and hemodynamic instability appear within 4 hours of ingestion. Pulmonary edema and pulmonary aspiration, consumption coagulopathy with decreased platelets, elevated serum transaminases, and persistent hemodynamic instability can develop subsequently. A high blood endosulfan level and initial hypotension indicate poor prognosis. The doses required to produce death are relatively large, and reports of death in humans were found only in cases of accidental or intentional ingestion of large quantities of endosulfan-containing pesticides. Therefore, it is likely that the risk of death is very small under conditions of long-term, low-level exposure either from ingestion of contaminated food or water (endosulfan is not readily water soluble) or inhalation of endosulfan dusts or mists.

The oral LD_{50} values reported for technical-grade endosulfan vary depending on the species, sex, formulation tested, vehicle used, and nutritional status of the animal (Boyd and Dobos 1969; Boyd et al. 1970; Gupta 1976; Gupta and Gupta 1979; Hoechst 1990; WHO 1984). Outward signs of acute endosulfan toxicity are similar among species and are generally associated with endosulfan's effects on the central nervous system. Symptoms include hyperexcitability, dyspnea, decreased respiration, and fine tremors followed by tonic-clonic convulsions (Boyd and Dobos 1969; Boyd et al. 1970; Ceron et al. 1995; Gilbert and Mack 1995). Deaths have also been reported in rats after intermediate-duration

exposure (Gilbert 1992) and in female mice after chronic-duration exposure (Hack et al. 1995). Although the specific cause of death was not discussed in the Hack et al. (1995) study, it does not appear that neurological effects played any role. Mice generally appear to be more sensitive to the lethal effects of endosulfan than rats (Gupta 1976; Gupta et al. 1981). Female rats are more sensitive to the lethal effects of endosulfan than male rats (Gupta 1976; Hoechst 1990).

Protein deficiency enhances the lethal effects of endosulfan in Wistar rats (Boyd and Dobos 1969; Boyd et al. 1970). Thus, humans consuming protein-deficient diets may represent a sensitive subpopulation (see Section 2.9).

Systemic Effects.

Respiratory Effects. No reports were located indicating that endosulfan is a significant respiratory irritant. However, respiratory effects (e.g., hypoxia, dyspnea, and cyanosis) have been observed in humans and animals following acute, high-level exposure to endosulfan (Blanco-Coronado et al. 1992; FMC 1958; Gupta and Chandra 1975; Hoechst 1970, 1983a, 1989b; Shemesh et al. 1988; Terziev et al. 1974). Since these respiratory effects have been reported only at doses that produce marked neurotoxicity and/or were lethal, it is likely that the effects observed in humans and animals following acute, high-level exposure are a result of disruption of the central nervous system control of respiratory activity. The possibility that endosulfan has a direct toxic effect on the respiratory tissues at these high doses cannot, however, be ruled out. It is not likely that humans exposed to low levels of endosulfan will experience adverse respiratory effects.

Cardiovascular Effects. Cardiovascular effects, such as tachycardia and hypertension, followed by cardiogenic shock, increased cardiac output, and an increase in total peripheral resistance, have been observed in humans and animals following acute, high-level exposure to endosulfan (Anand et al. 1980b, 1981; Shemesh et al. 1988). A more recent study reported severe hypotension followed by disseminated intravascular coagulation and cardiogenic shock in a woman who eventually died after ingesting endosulfan (Blanco-Coronado et al. 1992). Lo et al. (1995) reported that autopsy of a man who died after ingesting endosulfan showed cardiomegaly with congestive heart failure. In addition, systemic congestion consistent with acute heart failure has been observed in short-term, high-dose studies in animals (Gupta and Chandra 1977; Hoechst 1970). Histopathological changes in the hearts of animals have been observed in acute-duration, high-level exposures to endosulfan (Hoechst 1985c; Terziev et al. 1974). Also, calcification of the heart and blood vessels as a result of parathyroid hyperplasia secondary

to kidney disease was observed in male rats in a chronic duration study that used relatively high doses of endosulfan (NCI 1978). The available information does not suggest that endosulfan has a direct toxic effect on the cardiovascular system. These effects are only seen at doses that evoke convulsions, indicating that the cardiovascular effects are a result of increased central sympathetic nervous system activity. Therefore, it is not likely that humans exposed to low levels of endosulfan will experience adverse cardiovascular effects.

Gastrointestinal Effects. Nausea, gagging, vomiting, and/or diarrhea in humans and animals have been reported following acute, high-level oral exposure to endosulfan (Blanco-Coronado et al. 1992; Ceron et al. 1995; FMC 1958, 1959a; Hoechst 1989b; Singh et al. 1992; Terziev et al. 1974). Gross pathologic examination of gastrointestinal tissues from several studies have shown adverse effects (Boyd et al. 1970; FMC 1958; Hoechst 1970, 1989b) indicating that endosulfan may have a direct toxic effect on these tissues. However, a neurologic component of the emesis and diarrhea cannot be eliminated. Because these effects have been observed only following acute-duration, high-level exposures to endosulfan, it is unlikely that humans exposed to low levels of endosulfan will experience adverse gastrointestinal effects. Long-term studies in animals have provided no evidence of adverse gastrointestinal effects in rats, mice, or dogs (FMC 1959a, 1959b, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989a, 1989c).

Hematological Effects. Adverse hematological effects were reported in a group of six subjects who acutely accidentally or purposely consumed unknown, but assumed high, amounts of endosulfan (Blanco-Coronado et al. 1992). Five patients showed diminution of the platelets in circulating blood, consistent with consumption coagulopathy. One woman, who eventually died, developed disseminated intravascular coagulation along with thrombi of multiple vessels, including the aorta and the pulmonary artery system.

In animals, treatment-related effects on the hematopoietic system (decreases in hemoglobin, red cell count, mean corpuscular hemoglobin concentration, and/or packed cell volume) have been noted in a few studies following oral exposure to endosulfan (Ceron et al. 1995; Das and Garg 1981; Hoechst 1985a; Siddiqui et al. 1987b). In two cases, the effects on the hematopoietic system were observed following acute-duration, high-level exposure to endosulfan (Ceron et al. 1995; Siddiqui et al. 1987b), and in another study, the effects were observed at very low doses only following a pretreatment period of protein depletion (Das and Garg 1981). Only one study showed effects consistent with anemia following longer-duration oral exposure to moderate levels of endosulfan (Hoechst 1985a). However, several studies were located that do not indicate significant changes in these parameters following intermediate- or chronic-

duration oral exposure to endosulfan (Das and Garg 1981; FMC 1959a, 1959b, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989a, 1989c). In addition, one study indicated an increase in red cell count (Dikshith et al. 1984). Mixed results were also obtained in studies examining the effects of dermal exposure to endosulfan (Dikshith et al. 1988; Hoechst 1985c, 1985d). Thus, the available studies suggest that endosulfan can induce adverse hematological effects if sufficiently high doses are consumed. Protein deficiency or other unidentified stressors may enhance the anemia-inducing effect of endosulfan.

In vitro studies support the ability of endosulfan to adversely affect the red blood cell. Marked damage to human red blood cell membranes has been reported to occur *in vitro* at endosulfan concentrations as low as 1 ppb (in the medium) (Daniel et al. 1986). Fresh red blood cells were suspended in 1% normal saline and incubated for 60 minutes with various concentrations of endosulfan in ethanol. The red blood cells were examined immediately after incubation. The effects observed included crenation, threading, pitting of the surface, and loss of cellular outline and cell fusion. Cellular damage and altered cell morphology were accompanied by increased cell membrane permeability, as evidenced by the release of plasma hemoglobin into the test media. In addition, α-endosulfan, but not the β-isomer, was a strong inhibitor of the calcium transport ATPase of human erythrocyte membranes, suggesting that the chemical may be incorporated into membranes, thus impairing enzyme activity (Janik and Wolf 1992). Increased fragility and permeability of red blood cell membranes have also been observed in red blood cells obtained from cats that received a single intravenous dose of 3 mg/kg (Misra et al. 1982). Decreased erythrocyte Na^+, K^+-ATPase activity was observed by Kiran and Varma (1988) following acute oral administration of 12.5 mg/kg/day of endosulfan to rats, suggesting that endosulfan may alter cellular ion permeability by decreasing the activity of this enzyme.

Musculoskeletal Effects. Very limited data were available regarding the effects of endosulfan on the musculoskeletal system. However, the available animal data did not indicate that this system is adversely affected following either inhalation or oral exposure to endosulfan (FMC 1965, 1967; Hoechst 1984b, 1984c, 1988b, 1989a, 1989c). Thus, persons exposed to endosulfan would not be expected to experience adverse effects on the musculoskeletal system.

Hepatic Effects. Elevated serum transaminases (AST, ALT) were seen in a patient two days after ingesting an unknown amount of endosulfan mixed in with food (Blanco-Coronado et al. 1992). Eight days after admission to the hospital, the patient died, and postmortem examination revealed dilation and congestion of hepatic sinusoids. In another fatal case, postmortem examination revealed centrilobular congestion and slight prominence of the bile canaliculi (Lo et al. 1995). Studies using experimental

animals indicate that endosulfan affects the liver following oral, dermal, and parenteral exposure. The effects seen include an increase in liver weight and/or induction of microsomal enzymes (Anand et al. 1980b; Ansari et al. 1984; Banerjee and Hussain 1986, 1987; Das and Garg 1981; Den Tonkelaar and Van Esch 1974; Dikshith et al. 1984; Gupta and Chandra 1977; Gupta and Gupta 1977a; Hoechst 1984a; Kiran and Varma 1988; Misra et al. 1980; Siddiqui et al. 1987a). Although histopathological changes have generally not been reported to accompany the enzyme induction and increase in liver weight discussed above, Gupta and Chandra (1977) observed hepatic lesions that included moderate-to-severe inflammation, dilation of the sinusoids, necrosis, and Kupffer cell hyperplasia in rats administered 5 or 10 mg/kg/day of endosulfan by gavage for 15 days. Increased serum and liver transaminases activities, suggestive of liver damage, were reported in rabbits after a single endosulfan dose of 15.1 mg/kg (Ceron et al. 1995) and in rats after doses of 3 mg/kg/day in the food for 30 days (Paul et al. 1995). Based on results from *in vitro* studies, some have suggested that the cytotoxic effects of endosulfan in the liver are related to its ability to uncouple oxidative phosphorylation and inhibit electron transport (Dubey et al. 1984; Narayan et al. 1985a, 1985b).

The biological significance of the adaptive changes discussed above is not known since these effects have not been observed in most chronic studies (FMC 1959a, 1967; Hack et al. 1995; Hoechst 1988b, 1989a; NCI 1978). However, increased serum alkaline phosphatase in dogs consuming endosulfan for 1 year in the absence of detectable effects on liver weight, function, or microscopic damage (Hoechst 1989c) and an increased incidence of hydropic hepatic cells in rats consuming endosulfan for 2 years (FMC 1959b) have been observed in chronic-duration studies. Therefore, the potential exists for adverse hepatic effects in humans following long-term exposure to sufficiently high levels of endosulfan. A chronic-duration oral MRL was derived based on the observation of increased serum alkaline phosphatase in dogs (Hoechst 1989c). Also, as seen in the cases reported by Blanco-Coronado et al. (1992), short-term exposure to very high levels of endosulfan may cause adverse effects on the livers of humans.

Renal Effects. Hemorrhage of the medullary layer of the kidneys was observed in an early report of three fatal cases of acute oral poisoning with endosulfan (Terziev et al. 1974). More recent studies have reported acute renal failure after ingestion of endosulfan as a major contributing cause of death in two individuals; in both cases, postmortem examination showed extensive tubular necrosis (Blanco-Coronado et al. 1992; Lo et al. 1995). Neither case discussed the possible mechanism of endosulfan-induced acute renal failure, but in one case, the authors of the report indicate that the renal lesions may relate to sepsis and shock (Blanco-Coronado et al. 1992). Ingested doses were not determined in any of these cases, and

it is not totally clear that the effects observed at autopsy were a direct result of endosulfan exposure, although based on results from acute animal studies, it seems likely.

There have been no reports of renal toxicity associated with acute inhalation exposure to endosulfan in laboratory animals. However, acute oral and dermal exposure to endosulfan has been reported to cause damage to the kidneys of rats, rabbits, and dogs (FMC 1958, 1980a; Gupta and Chandra 1975; Hoechst 1970; Terziev et al. 1974). This is consistent with acute oral exposure in humans. Intermediate-duration ingestion of endosulfan by rats has also been reported to result in renal changes (Dikshith et al. 1984; Gupta and Chandra 1977; Hoechst 1984a, 1985a). Some of these changes are manifestations of renal toxicity, whereas for others, it is unclear whether the effects observed represent a toxic effect. For example, clearly toxic responses included congestion, shrunken glomerular tufts, thickened Bowman's capsules, focal degeneration or necrosis of the epithelial lining of the kidney tubules, protein aggregates in the lumen of renal tubules, and eosinophilic droplets in cells of the proximal convoluted tubules (Gupta and Chandra 1975, 1977; Hoechst 1985a). In contrast, yellowish discoloration of the cells of the proximal convoluted tubules and granular/clumped pigment in the cells of the straight portions and/or proximal convoluted tubules may either represent storage of endosulfan in the kidney or renal pathology. Although the Hoechst (1987) study indicated that the yellow discoloration and increases in kidney weight may be a reflection of lysosomal sequestration of endosulfan in the cells of the renal tubules, tests assessing renal performance were not conducted. Thus, the significance of these changes remains unclear. Chronic ingestion of endosulfan has also resulted in renal toxicity in male rats (FMC 1959b; Hack et al. 1995; Hoechst 1989a; NCI 1978). Thus, individuals exposed to sufficiently high levels of endosulfan for either acute, intermediate, or chronic durations may be at risk for compromised renal function and possible injury.

Endocrine Effects. No information was located regarding endocrine effects in humans following exposure to endosulfan. In animals, with one exception, routine and/or microscopic examination of endocrine glands following inhalation (Hoechst 1984c) or oral exposure (FMC 1965, 1967; Gupta and Gupta 1977a; Hoechst 1984a, 1988b, 1989a, 1989c) to endosulfan for various durations revealed no significant treatment-related effects. The exception is the observation of degranulation of the β-cells of the islets of Langerhans of the pancreas in rats administered a single oral dose of 1 mg of endosulfan/kg (Barooah et al. 1980). This effect, however, was not observed after the same dose was administered daily for five days. Both administration protocols caused dilation of the blood vessels of the islets of Langerhans. Microscopic alterations of the adrenals were observed in rabbits (Gupta and Chandra 1975) and rats (Hoechst 1985c) after dermal application of endosulfan; however, these were lethal or near-lethal

dermal doses of endosulfan. Adverse endocrine effects secondary to chronic renal failure were observed in rats treated chronically with endosulfan in the diet (NCI 1978). The effects consisted of parathyroid hyperplasia and calcification of tissues.

In recent years, concern has been raised that many industrial chemicals, endosulfan among them, are endocrine-active compounds capable of having widespread adverse effects on reproductive health of humans and wildlife (Daston et al. 1997; Safe et al. 1997). Numerous studies have examined the possibility that endosulfan might be an endocrine-disrupter. The overall evidence is mixed. A variety of *in vitro* assays indicate that endosulfan has weakly estrogenic properties at exposure levels of approximately 5–50 µM (the estrogenic effect of endosulfan treatment was approximately 30–85% of that seen after treatment with approximately 0.01 µM 17β-estradiol) and is generally cytotoxic at about 100 µM (Andersen et al. 1999; Legler et al. 1999; Ramamoorthy et al. 1997; Soto et al. 1994, 1995; Vonier et al. 1996; Wade et al. 1997), while other *in vitro* assays apparently contradict these findings, showing no endocrine disruptive activity at similar exposure levels (Andersen et al. 1999; Arcaro et al. 1998; Hsu et al. 1998; Shelby et al. 1996). Endosulfan was not estrogenic in female animals as indicated by lack of changes in relative uterine weight in the following three *in vivo* assays: ovariectomized female rats orally administered 1.5 mg/kg/day for 30 days (Raizada et al. 1991); immature female rats administered 3 mg/kg/day by intraperitoneal injection for 3 days (Wade et al. 1997); immature female mice acutely exposed by subcutaneous injection for 3 days at up to 10 mg/kg/day (Shelby et al. 1996). However, in male rats, altered testicular testosterone was seen after both acute- and intermediate-duration oral exposures to $7.5 mg/kg/day (Singh and Pandey 1989, 1990). Further details of studies that evaluate whether endosulfan is an endocrine disrupter are provided in Section 2.6 (Endocrine Disruption). Overall, there is only weak evidence to suggest that levels in the environment to which the general population is exposed could induce adverse endocrine effects.

Dermal Effects. There have been no reports of adverse dermal effects associated with exposure to endosulfan in humans. When tested in farmers, endosulfan did not cause contact dermatitis (Schuman and Dobson 1985). Studies in experimental animals have shown that dermal exposure to endosulfan is only slightly to moderately irritating at relatively high doses (Hoechst 1983b, 1985c, 1985d, 1989b; Industria Prodotti Chimici 1975).

Ocular Effects. An aqueous solution of endosulfan was found to be nonirritating to the eyes of rabbits (Gupta and Chandra 1975), and ophthalmologic examination of the eyes of rats, mice, and dogs following intermediate- or chronic-duration oral exposure revealed no adverse effects (Hack et al. 1995; Hoechst

1984b, 1985a, 1985b, 1988b, 1989a, 1989b, 1989c). However, it has been reported that chronic intraperitoneal administration of endosulfan to rabbits induced widespread damage to the ocular tissues (Anand et al. 1987). The authors hypothesized that the ocular effects associated with endosulfan may be a result of prolonged hypertension (although no data on blood pressure were presented, and there is no other information to indicate that chronically administered endosulfan induces hypertension) or an endosulfan-induced vitamin A deficiency (which was observed in this study). Although the rabbit may represent a uniquely sensitive species, the possibility that long-term exposure of persons at hazardous waste sites to endosulfan may result in adverse effects on ocular tissues cannot be eliminated.

Body Weight Effects. No information was located regarding body weight effects in humans following exposure to endosulfan. One study in rats reported decreased body weight gain following intermediate-duration nose-only exposure to 2 mg/m^3 aerosolized technical endosulfan (Hoechst 1984c). Numerous studies monitored body weight in animals following oral exposure. It is apparent that doses of endosulfan that decreased survival and/or caused frank toxicity also decreased body weight gain. This was demonstrated in an acute-duration study in rabbits (Ceron et al. 1995) and an intermediate-duration study in rats (Gupta and Chandra 1977) and in a chronic study in rats (NCI 1978). Food consumption was significantly depressed in the study in rabbits, but no information was provided in the rat studies. Interestingly, in a 24-month feeding study in rats and mice, body weight gain was significantly reduced in male mice and in male and female rats even though the authors reported that food and water consumption were not significantly altered in either species (Hack et al. 1995). No explanation was provided for this finding, and its significance for human health is unknown.

Metabolic Effects. Severe metabolic acidosis with high anion gap and hyperglycemia were reported in humans acutely poisoned by ingestion of endosulfan (Blanco-Coronado et al. 1992; Lo et al. 1995). In the cases reported by Blanco-Coronado et al. (1992), mitigation of the initial convulsive activity also corrected the metabolic acidosis, which according to the authors suggested that anoxia due to convulsions may be the primary cause of the metabolic acidosis. Studies in rats suggest opposing effects on circulating glucose levels depending on dose. After a single moderate dose of endosulfan, degranulation of the β-cells of the islets of Langerhans of the pancreas (indicating release of insulin) and decreased serum glucose have been observed (Barooah et al. 1980). After higher doses of endosulfan, decreased hepatic glycogen and increased serum glucose have been observed (Chatterjee et al. 1986; Garg et al. 1980; Kiran and Varma 1988). It should also be noted that the studies that reported hyperglycemia and decreased hepatic glycogen also reported frank neurotoxicity manifested as convulsive activity. It may well be, as pointed out by Kiran and Varma (1988), that the hyperglycemia is due to the mobilization of

glucose from store sites (like the liver) triggered by the energy demands of a drastic increase in muscle activity that follow high doses of endosulfan. The limited information available suggests that exposure of persons to endosulfan in dosages high enough to cause muscle tremor or seizures, may result in metabolic acidosis and hyperglycemia. Daily exposure of the general population to levels of endosulfan commonly found in food stuff is not expected to induced adverse metabolic effects.

Other Systemic Effects. Decreased food consumption has been reported in acute- and intermediate-duration oral studies in animals administered technical endosulfan by gavage. However, in the acute-duration study in rabbits endosulfan caused frank toxicity, including seizure activity, and was lethal to 5 of 7 rabbits (Ceron et al. 1995). In the study in rats (Paul et al. 1994), while there were no severe neurotoxic effects, spontaneous motor activity was significantly increased in the treated animals, and this may have contributed to the reduced food intake. In one chronic-duration feeding study in rats and mice treatment with endosulfan did not result in significant alterations in food or water consumption, but body weight gain was reduced in the animals (Hack et al. 1995). Whether this suggests altered food utilization remains unclear, and its significance to human health is unknown.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after exposure to endosulfan. However, studies in rats indicate that both humoral and cellular immune responses (e.g., serum antibody titer to tetanus toxin; IgG, IgM, and γ-globulin levels; MMI and LMI) are depressed by oral exposure to endosulfan at doses that do not induce any other overt signs of toxicity (Banerjee and Hussain 1986, 1987). Based on the observation of depressed cellular and humoral responses following oral exposure of rats to 0.9 mg/kg/day for 22 weeks and the absence of this effect at 0.45 mg/kg/day (Banerjee and Hussain 1986), an intermediate-duration MRL of 0.005 mg/kg/day was derived. Mice administered daily intraperitoneal injections of approximately 0.8 mg/kg/day in 10 administrations over a period of 30 days (daily dose level was ambiguously reported) also showed impaired humoral (bovine serum albumin antibody titre) and cell-mediated (LMI and phagocytic activity) immune responses (Bhatia et al. 1998). These results demonstrate that immunotoxicity may be a more sensitive end point of endosulfan-induced toxicity than other end points (e.g., neurotoxicity) and that humans may be at risk for adverse immune effects following exposure to endosulfan.

The observed immunosuppressive effect could be due either to a general effect on the animal's physiological condition, hormonal function, nutritional status, or hepatic metabolism of other endogenous and immunoregulatory substances or an effect on lymphoid cells, lymphoid cell distribution,

immunoglobulin metabolism, T-cell/B-cell-macrophage cooperation, or macromolecular biosynthesis (Banarjee and Hussain 1986). Serum complement (a group of serum proteins believed to be involved in immunopathology and immunoregulation) may be involved in some immunological alterations induced by endosulfan, as proposed by Das et al. (1988). They found that endosulfan activated serum complement *in vitro* by the alternative pathway. Complement activation has been shown to occur in association with many pathological conditions, including allergic diseases. In summary, sensitive indicators of both humoral and immune function have suggested altered immunocompetence at doses of endosulfan that have not been previously shown to cause toxicity.

Neurological Effects. The most prominent signs of acute exposure to high concentrations of endosulfan in humans are hyperactivity, tremors, decreased respiration, dyspnea, salivation, and tonic-clonic convulsions. These effects have been observed in cases of occupational exposure as well as following intentional or accidental ingestion of large amounts of endosulfan. Autopsies performed in three cases of lethal exposure to endosulfan in humans revealed brain edema (Terziev et al. 1974), but a more recent study found no hemorrhagic areas in the brain of a patient who also died after ingestion of endosulfan (Blanco-Coronado et al. 1992). One year after an attempted suicide by ingestion of an endosulfan-containing pesticide, the mental activity (presumably psychomotor function) of a 20-year-old man was still severely impaired, and he required medication to control his seizures (Shemesh et al. 1988). Long-term brain damage has also been associated with occupational endosulfan intoxication (Aleksandrowicz 1979). These case reports suggest that long-term brain damage can occur following exposure to high concentrations of endosulfan in humans. The brain damage may have been a result of a direct action of endosulfan on the brain tissue or the hypoxia that accompanied seizures. However, interpretation of these studies is difficult because limited information was presented regarding neuropsychiatric status before exposure to endosulfan. It is possible that not all of the defects observed post-ingestion are attributable to the pesticide.

Signs of acute endosulfan intoxication similar to those reported in humans have been observed in animals. Also, cerebral congestion and edema is often observed at necropsy in animals that die following acute ingestion of endosulfan (Boyd and Dobos 1969; Boyd et al. 1970; Terziev et al. 1974). The severe central nervous system effects described above have not been described in many intermediate or chronic-duration ingestion studies of endosulfan in experimental animals. This may reflect lack of careful observation of the animals, administration of relatively low doses of endosulfan, or increased tolerance to endosulfan.

Perhaps one of the most important findings in animal studies in recent years regarding the neurological effects of endosulfan is that low doses of endosulfan reduced the threshold for seizures produced by electrical stimulation in kindled animals (Gilbert and Mack 1995). Also, the increase in seizure susceptibility was long-lasting and transferred positively to electrical kindling up to 1 month following cessation of treatment. Keeping in mind that the endosulfan doses administered to the animals in these studies are well above those typically found in the environment, these results indicate that humans with a predisposition to seizure disorders through hereditary or environmental causes may be at higher risk to the adverse effects of endosulfan and related chemicals (Gilbert and Mack 1995). Recent studies in animals have also found that administration of endosulfan can induce changes in the levels of various neurotransmitter in different brain areas (Lakshmana and Raju 1994) and alter learning and memory processes (Paul et al. 1994, 1995). The possible mechanisms by which endosulfan can induce some of the neurotoxic effects that have been observed are briefly discussed in Section 2.4.2.

In summary, the frank neurotoxic effects of endosulfan are apparent only after acute ingestion of relatively high doses in animals. However, long-term decreased psychomotor function, possibly resulting from acute endosulfan exposure, have been reported by two authors (Aleksandrowicz 1979; Shemesh et al. 1988). Such effects cannot be easily measured in animals. Hence, the fact that long-term neurotoxic effects have not been observed in animals does not mean that such effects cannot occur in humans. However, no information was located that indicated that persons exposed to low levels of endosulfan might experience any neurotoxicity.

Reproductive Effects. No studies were located regarding reproductive effects in humans after exposure to endosulfan. Three studies were available regarding the effects of oral endosulfan exposure on reproductive performance in animals, and all yielded negative results (Dikshith et al. 1984; Hoechst 1982, 1984a). In contrast, studies that evaluated the toxicity of endosulfan on reproductive organs rather than on reproductive function showed evidence of the ability of endosulfan to adversely affect male reproductive organs (Gupta and Gupta 1977a; Khan and Sinha 1996; NCI 1978; Singh and Pandey 1989; Singh and Pandey 1990; Sinha et al. 1997). Male mice showed a significant reduction in sperm counts after 35 days of treatment with 3 mg/kg/day (Khan and Sinha 1996), and similar effects were seen adult male rats treated with 5 mg/kg/day for 70 days (Sinha et al. 1995) or in young male rats treated for 90 days (Sinha et al. 1997). Neither one of these two studies examined reproductive performance. In the Khan and Sinha (1996) study, simultaneous intraperitoneal administration of different doses of vitamin C reduced the effects of endosulfan in a dose-related manner. A higher-dose study showed that male rats given 10 mg/kg/day for 15 days had increased weight of the testes with marked degenerative changes in

the seminiferous epithelium of the tubules, but no examinations were conducted on spermatozoa production or the reproductive performance of the animals (Gupta and Gupta 1977a). Singh and Pandey (1989, 1990) reported effects on testosterone production in male rats after exposure to endosulfan (at approximately 7.5–10 mg/kg/day), which may possibly lead to reproductive toxicity. Wilson and LeBlanc (1998) recently showed that short-term administration of endosulfan to female mice increased the clearance of testosterone, but this was associated with only a small, nonsignificant decrease in serum testosterone levels. Histopathological effects (a dose-related atrophy characterized by testicular degeneration and necrosis) were noted in the reproductive organs of male rats fed a diet that provided approximately 48 mg endosulfan/kg/day for 74 weeks (NCI 1978); the no-effect-level in that study was 20 mg/kg/day. Chronic-duration exposure studies in rats and mice with doses <5 mg/kg/day provided no evidence of gross or microscopic alterations in the reproductive organs, but the scope of the microscopical evaluation was not provided (Hack et al. 1995). A recent study with human sperm *in vitro* showed that concentrations of technical endosulfan as low as 0.1 nM strongly inhibited the acrosome reaction (AR, an essential fertilization event) initiated by glycine; 1 nM also inhibited the acrosomal reaction initiated by progesterone (Turner et al. 1997). Chloride channels activated by GABA are involved in the AR. Turner et al. (1997) pointed out that although their results were striking because of the low concentration of endosulfan at which the inhibition occurred, there is yet no established link between human or wildlife infertility, the AR inhibitory levels of endosulfan, and the levels of endosulfan found in the environment. *In vitro* cultures of preimplantation hybrid mouse embryos showed significantly increased incidence of mice with altered preimplantation development after cultivation in serum of rats that had been exposed intraperitoneally with 10 mg/kg of the powder or 200 mg/kg in microcapsular form (Popov et al. 1998a).

In summary, although the available reproductive studies indicate that endosulfan has no adverse effects on reproductive performance in animals, severe adverse effects on male reproductive organs have been seen in rats and mice. This apparent discrepancy needs to be resolved with further research. Endosulfan may potentially cause reproductive toxicity in humans.

As discussed in the Endocrine Effects section, endosulfan has shown weak estrogenic properties in some *in vitro* assays, but no such properties could be confirmed in studies *in vivo*.

Developmental Effects. No studies were located regarding developmental toxicity in humans after exposure to endosulfan. In animals, the evidence is inconclusive. Studies of gestational exposure found increased skeletal variations and resorptions at endosulfan doses of 5 mg/kg/day or greater, which also caused maternal death (Gupta et al. 1978). Another study found increased skeletal variations and

decreased birth weight and length at doses of 6 mg/kg/day, which also induced maternal toxicity (FMC 1980b). Furthermore, in the FMC (1980b) study, replacement of animals during and after the study made it difficult to interpret the data (IRIS 2000).

A study of exposure of rats to endosulfan prior to and during gestation, as well as during lactation, reported fetal toxicity (decreased litter weights during lactation), but only in the presence of maternal toxicity (Hoechst 1982). A similar subsequent study showed lower fetal weights during lactation in the absence of observed maternal toxicity (Hoechst 1984a), but because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, the decrease in litter weight was not considered to be treatment-related (IRIS 2000).

A study conducted in rat pups in which the animals were treated intraperitoneally with 1 mg of technical endosulfan/kg/day for 25 days beginning at 1 day of age found a significant increase in the binding of serotonin to frontal cortical membranes (Zaidi et al. 1985). This increase correlated well with an increase in aggressive behavior. In contrast, exposure of adults to 1 mg/kg for 30 consecutive days or 3 mg/kg for 15 days did not induce significant changes in the binding or in aggressive behavior (Seth et al. 1986). Without further elaboration, Seth et al. (1986) suggested that neonates are more sensitive because serotonergic receptors develop postnatally (see also Section 2.6). Based on data from existing studies in animals, there is inconclusive evidence to characterize endosulfan as a potential developmental toxicant in humans. *In vitro* cultures of postimplantation rat embryos showed significantly increased incidences of embryolethality and developmental abnormalities both after direct exposure to endosulfan in powder or microcapsular form, and after cultivation in serum of rats that had been exposed intraperitoneally with either the powder or microcapsular form (Popov et al. 1998a).

Genotoxic Effects. Endosulfan has been evaluated for genotoxicity in a variety of *in vivo* and *in vitro* assays. As summarized in Tables 2-4 and 2-5, the results of these assays have been positive and negative, but the majority of mutagenicity tests reported positive results. Certain studies were unsatisfactory, as indicated below.

DNA damage in mononuclear leukocytes was significantly increased in agricultural workers after occupational exposure to a pesticide mixture including endosulfan, although the contribution of endosulfan to the observed effect is uncertain (Lebailly et al. 1998). Both positive (Falck et al. 1999) and negative (Scarpato et al. 1996a, 1996b; Venegas et al. 1998) results were obtained in peripheral

Table 2-4. Genotoxicity of Endosulfan *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Mouse spermatogonial cells	Chromosomal aberrations	+	Usha Rani and Reddy 1986
Rat spermatogonial cells	Chromosomal aberrations	–	Dikshith and Datta 1978; Dikshith et al. 1978
Rat spermatogonial cells	Aberrant metaphases	+	Dikshith et al. 1978
Rat bone marrow cells	Chromosomal aberrations	–	Dikshith and Datta 1978; Dikshith et al. 1978
Rat bone marrow cells	Aberrant metaphases	+	Dikshith et al. 1978
Mouse bone marrow polychromatic-erythrocyte assay (micronucleus test)	Micronuclei	–	Usha Rani et al. 1980
Mouse bone marrow	Chromosomal aberrations	+	Kurinnyi et al. 1982
Hamster bone marrow	Chromosomal aberrations	+	Dzwonkowska and Hubner 1986
Mouse bone marrow	Aberrant metaphases	+	L'Vova 1984
Insect systems:			
Drosophila melanogaster (sex-linked recessive lethal test)	Recessive lethal mutation	+	Velazquez et al. 1984
D. melanogaster	Sex-chromosome loss	+	Velazquez et al. 1984

+ = positive results; – = negative results

Table 2-5. Genotoxicity of Endosulfan *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100	Gene mutation	–	–	Pednekar et al. 1987
S. typhimurium TA89, TA100, TA1535, TA1537, TA1538	Gene mutation	No data	–	Moriya et al. 1983
S. typhimurium TA98, TA100, TA1535, TA1978	Spot test	No data	No data	Dorough et al. 1978
S. typhimurium TA 1535/pSK 1002	umu gene expression	No data	+	Chaudhuri et al. 1999
Escherichia coli WP hcr	Gene mutation	No data	–	Moriya et al. 1983
E. coli K12	Gene mutation	No data	+	Chaudhuri et al. 1999
E. coli WP2s	prophage λ induction	No data	+	Chaudhuri et al. 1999
Eukaryotic organisms:				
Saccharomyces cervisiae	Miotic cross over	No data	–	Yadav et al. 1982
S. cerevisiae D7	Reverse mutation	No data	+	Yadav et al. 1982
S. cerevisiae D7	Mitotic gene conversion	No data	+	Yadav et al. 1982
S. cerevisiae D7	Aberrant colonies	No data	+	Yadav et al. 1982
S. cerevisiae D7	Mitotic gene conversion	No data	–	L'Vova 1984
S. cerevisiae T2 (PG-155)	Mitotic recombination	No data	+	L'Vova 1984
Mammalian cells:				
Cultured human lymphocytes	Mitotic recombination Aberrant metaphases	No data	–	L'Vova 1984
Cultured human lymphocytes	Sister chromatid exchange	+	+	Sobti et al. 1983
Human liver hepatoblastoma	DNA adducts	+	No data	Dubois et al. 1996

Table 2-5. Genotoxicity of Endosulfan *In Vitro (continued)*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Fetal rat hepatocytes	DNA adducts	+	No data	Dubois et al. 1996
Rat hepatocyte culture	Unscheduled DNA synthesis	No data	–	Hoechst 1984c

+ = positive results; – = negative results; DNA = deoxyribonucleic acid

lymphocyte micronucleus studies in workers who applied various pesticides, including endosulfan. No increase in chromosomal aberrations or sister chromatid exchanges was seen in greenhouse workers in Italy who were exposed to complex mixtures of pesticides that included endosulfan (Scarpato et al. 1996a, 1996b, 1997). The mixed results in human genotoxicity assays should be treated with caution because coexposure to a variety of other chemicals occurred in each study and the exposure levels of endosulfan were not reported.

The induction of genotoxic effects in animals following *in vivo* exposure to endosulfan has been evaluated in the chromosomal aberration test in somatic and germinal cell systems with rats (Dikshith et al. 1978), mice (Kurinnyi et al. 1982; Usha Rani and Reddy 1986), and hamsters (Dzwonkowska and Hubner 1986), as well as in the bone marrow micronucleus test with mice (Usha Rani et al. 1980), and in the sex-linked recessive lethal mutation test with *Drosophila* (Velazquez et al. 1984). Endosulfan enhanced chromosomal aberrations in mouse spermatocytes 60 days posttreatment (Usha Rani and Reddy 1986), in mouse bone marrow (Kurinnyi et al. 1982) and in hamster bone marrow (Dzwonkowska and Hubner 1986), but the pesticide failed to induce chromosomal aberrations in the bone marrow and spermatogonial cells of rats (Dikshith and Datta 1978; Dikshith et al. 1978). However, it is not known how soon after treatment the animals were killed, and as shown in the mouse studies (Usha Rani and Reddy 1986), a latency period of 60 days was required to see chromosomal aberrations in spermatogonia. Endosulfan also increased the cytogenetic activity (aberrant metaphases) of mouse bone marrow (L'Vova 1984). In rats, relatively significant changes in mitotic indices (decreased metaphases) in bone marrow and spermatogonial cells have been observed (Dikshith et al. 1978). Endosulfan did not induce micronuclei in mice (Usha Rani et al. 1980). Endosulfan was positive *in vivo* for the induction of sex-linked recessive lethals and sex-chromosome loss, which indicates that endosulfan is an efficient mutagen in *Drosophila* (Velazquez et al. 1984). The incidence of *in vitro* sister chromatid exchanges was increased at least 5-fold compared to controls in cultured postimplantation rat embryos both after direct exposure to endosulfan in powder or microcapsular form, and after cultivation in serum of rats that had been exposed intraperitoneally with either the powder or microcapsulated endosulfan (Popov et al. 1998a).

Endosulfan is toxic to yeast but is also mutagenic without activation (Yadav et al. 1982). *In vitro*, endosulfan induced reverse mutations and mitotic gene conversion and increased the percentage of aberrant colonies in *Saccharomyces cerevisiae* but did not induce mitotic cross-overs (Yadav et al. 1982). This indicates that endosulfan is capable of inducing chromosome breakage and loss. Endosulfan also

induced cytotoxic activity (significant increase in the number of crossover colonies) in the yeast strain *S. cerevisiae* T2 (deficient in repair system), but not in *S. cerevisiae* T1 (L'Vova 1984).

No mutagenic activity was demonstrated for the *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, TA1537, TA1538 without activation (Moriya et al. 1983; Pednekar et al. 1987) or for *Escherichia coli* WP2 without activation (Moriya et al. 1983). Endosulfan also tested negative in the *Salmonella* mutagenicity test with or without activation with S9 liver homogenate (Dorough et al. 1978). A forward mutation assay in *E. coli* K12 showed an endosulfan-induced increase in mutations from ampicillin-sensitive to ampicillin-resistant (Chaudhuri et al. 1999). Prophage λ was also induced by endosulfan in *E. coli*, and *umu* gene expression was induced by endosulfan exposure in *S. typhimurium* (Chaudhuri et al. 1999).

In cultured mammalian cells, endosulfan was reported positive in sister chromatid exchanges in human lymphoid cells exposed both with and without activation (Sobti et al. 1983) and in forward locus mutations in mouse lymphoma cells in the absence of S9 mix (McGregor et al. 1988). Endosulfan did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Hoechst 1984d). Negative results were also reported for UDS in human lung carcinoma (A 549) cells using liquid scintillation counting (Hoechst 1988d), but the study was inconclusive because the author did not present any evidence that DNA synthesis was inhibited, and high background levels compromised the sensitivity of the assay. In a more recent study, endosulfan was found to induce the formation of DNA adducts in both fetal rat hepatocytes and Hep G2 (human liver hepatoblastoma) cells (Dubois et al. 1996); this activity strongly correlated with high induction of CYP3A gene expression.

In summary, genotoxicity studies of endosulfan have provided evidence that this compound is mutagenic and clastogenic, and that it induces effects on cell cycle kinetics in two different mammalian species. However, some of these data may be suspect because some formulations of endosulfan have contained epichlorohydrin, a known genotoxic chemical, as a stabilizer (Hoechst 1990). It should be noted that humans may also be exposed to epichlorohydrin along with endosulfan.

Cancer. No reports of cancer in humans associated with exposure to endosulfan have been found. The carcinogenicity of endosulfan has been studied in chronic oral bioassays using rats (FMC 1959b; Hack et al. 1995; Hoechst 1989a; NCI 1978) and mice (Hack et al. 1995; Hoechst 1988b; NCI 1968, 1978). While early studies in experimental animals have limitations (e.g., poor survival, less-than-lifetime exposures, inadequate reporting of data, use of only one dose level, and use of doses that were possibly

less than the maximum tolerated dose) that render them inadequate for drawing definitive conclusions regarding the carcinogenicity of endosulfan, the later studies by Hack et al. (1995) and earlier ones by Hoechst (1988b, 1989a) show no evidence of increased neoplasms in rats or mice chronically exposed to endosulfan. Consumption of 3.8 mg/kg/day (females) or 2.9 mg/kg/day (males) by Sprague-Dawley rats for 2 years did not result in an increased incidence of any neoplastic lesion (Hack et al. 1995; Hoechst 1989a). Similarly, consumption of 2.86 mg/kg/day (females) or 2.51 mg/kg/day (males) by NMRI mice for 2 years resulted in no increase in neoplastic lesions in these animals (Hack et al. 1995; Hoechst 1988b).

Technical endosulfan as well as the α- and β-isomers showed promoting activity in a two-stage, altered hepatic foci bioassay in male rats; of the three compounds, α-endosulfan had the strongest promoting activity (Fransson-Steen et al. 1992). Because both the phenobarbital and methylcholanthrene-inducible forms of hepatic cytochrome P450-dependent monooxygenases were marginally induced by the chemicals, an involvement of cytochrome P450 in the tumor promoting activity of these chemicals was considered unlikely (Fransson-Steen et al. 1992). Instead, the authors suggested that endosulfan is a tumor-promoting agent acting by clonal expansion of initiated cells. One possible mechanism of tumor promotion is the inhibition of gap junctional intercellular communication and the possibility that endosulfan exhibits such property has been studied *in vitro*. Results from studies by Fransson-Steen and Warngard (1992) showed that in primary rat hepatocytes β-endosulfan is a more potent inhibitor of intercellular communication than α-endosulfan, but the two isomers had similar inhibitory potency in WB-Fischer 344 rat liver epithelial cells. The mechanism of inhibition of intercellular communication by endosulfan has not been elucidated, but results from studies in IAR 20 rat liver epithelial cells have suggested an effect on connexin 43, the main gap junction protein in this cell line (Kenne et al. 1994). Phosphorylation of connexins is one posttranslational alteration involved in regulation of gap junctional communication (Musil et al. 1990). In the IAR 20 cell line, endosulfan was found to increase slightly phosphorylation of connexin 43 initially during the assay, but longer exposure periods led to hypophosphorylation (Kenne et al. 1994). While these *in vitro* assays are useful in short-term detection of tumor promoters, the general biological significance of gap junctional intercellular communication in tumor promotion needs further clarification (Fransson-Steen and Warngard 1992).

2.6 ENDOCRINE DISRUPTION

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These compounds are derived from plants and are similar in structure and action as endogenous estrogen. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997a). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in humans after exposure to endosulfan.

In vivo studies in animals suggest that endosulfan may disrupt normal reproductive hormone levels in male animals, but that it is not an endocrine disrupter in females. Persistent depressed testicular testosterone was seen in male rats after intermediate duration oral exposures to endosulfan. In ovariectomized female rats, orally administered endosulfan did not induce normal development of female reproductive tissues, and in female mice and immature female rats, acute parenteral exposure to endosulfan did not affect several endocrine-related end points. *In vitro* studies have evaluated endosulfan for estrogen receptor (ER) and cytosolic protein binding affinity, ER-mediated reporter gene expression, estrogenic induction of cell proliferation, and alteration of relative abundance of active estradiol metabolites. Overall, *in vitro* evidence in favor of endosulfan estrogenicity indicates relatively weak potency compared to 17β-estradiol. Apparently contradictory results were reported in different studies for several of the assays, indicating that caution should be used in interpreting the collective *in vitro* results.

Significantly increased serum testosterone and decreased testicular testosterone were reported in male rats after a 7-day exposure to endosulfan using oral doses in the range of 7.5–10 mg/kg/day, but not at #5 mg/kg/day (Singh and Pandey 1989). However, results after a 15-day exposure were highly variable and frequently not dose-related, making interpretation of the significance of the study's results difficult. A subsequent study (Singh and Pandey 1990) indicated a dose-related decrease in testicular testosterone, and plasma testosterone, LH, and FSH in groups of male Wistar rats orally administered endosulfan at 0, 7.5, or 10 mg/kg/day for 15 or 30 days. In addition, activities of steriodogenic enzymes and testicular cytochrome P450-dependent monooxygenases were depressed after the 30-day exposure at $7.5 mg/kg/day. All of the effects from 30 days of exposure were reversible during a 7-day recovery period, except for decreased testicular testosterone, which remained depressed; no recovery period was utilized for the 15-day exposures.

In ovariectomized female rats, gavage administration of 1.5 mg endosulfan/kg/day for 30 days did not influence the relative weights or histology of the uterus, cervix, or vagina compared to ovariectomized control rats that did not receive endosulfan (Raizada et al. 1991). Rats in a positive control group received intraperitoneal injections of estradiol (dose not reported) and showed increased relative organ weights and normal development of female reproductive tissues compared to the untreated ovariectomized control rats. Organ weights and tissue development in rats administered both endosulfan and estradiol were not significantly different from those seen in rats that received estradiol alone. The Raizada et al. (1991) study results indicate that endosulfan was neither estrogenic nor anti-estrogenic with respect to the end points evaluated and under the conditions of this assay. Immature female rats intraperitoneally administered technical grade endosulfan at 3 mg/kg/day for 3 days showed no changes with respect to relative uterine and pituitary weights, uterine peroxidase activity, circulating thyroxine levels, or to levels of FSH, LH, TSH, prolactin, and growth hormone in the pituitary gland (Wade et al. 1997). As an extension of the same assay, endosulfan did not alter relative levels of ER or progesterone receptors compared to controls in crude uterine cytosol prepared from uterine tissue of the rats dosed intraperitoneally. Uterine weight in female mice was not affected by acute subcutaneous administration of technical grade endosulfan at up to 10 mg/kg/day for 3 days, whereas 17β-estradiol at up to 4 mg/kg/day gave a strong positive response (Shelby et al. 1996).

Catfish (*Clarias batrachus*) plasma vitellogenin levels were significantly decreased after $48 hours of exposure to 0.0015 mg/L of commercial-grade endosulfan (Chakravorty et al. 1992). Levels did not recover substantially with injections of various hormones, including estradiol. In rainbow trout,

endosulfan did not induce vitellogenin production at 9 days after a single intraperitoneal injection of 5 mg/kg in peanut oil (Andersen et al. 1999).

In vitro direct binding affinity of endosulfan for human ER was approximately 12,000-fold lower than 17β-estradiol and negligible for rabbit uterus ER. Endosulfan (60% α-isomer and 38% β-isomer) at 300 µM had approximately 20 times lower *in vitro* binding affinity for oviductal cytosolic binding proteins of yellow-bellied turtle (*Trachemys scripta*) and American alligator (*Alligator mississippiensis*) compared to 17β-estradiol at 1 µM (Crain et al. 1998). The results of these studies suggest that the relatively low binding affinity of endosulfan for ER may be somewhat offset by a relatively lower binding affinity for cytosolic proteins, producing a relatively greater bioavailability for interacting with intracellular steroid receptors than estradiol. Indeed, in a competitive ER-binding assay, endosulfan significantly inhibited both [3H]17β-estradiol binding to the estrogen receptor and progestin [3H]R5020 binding to the progesterone receptor using receptors prepared from alligator oviduct tissue (Vonier et al. 1996). However, in another competitive binding assay, neither of the endosulfan isomers either singly or in combination with dieldrin inhibited 17β-estradiol binding either to recombinant human ER at concentrations up to 10 µM (Arcaro et al. 1998) or to mouse uterine receptor (Shelby et al. 1996). Similarly, 17β-estradiol-induced foci formation in MCF-7 human breast cancer cells was neither inhibited nor stimulated by cotreatment with endosulfan (Arcaro et al. 1998).

ER-mediated reporter gene expression was related to endosulfan incubation concentration; in general, 100 µM induced gene expression, while mixed results were obtained at lower concentrations. Endosulfan induced human ER-mediated β-galactosidase (β-gal) activity at 100 µM in an estrogen-responsive reporter system in yeast, but not at #10 µM (Ramamoorthy et al. 1997). The endosulfan-induced yeast β-gal activity was about 32% of that induced by estradiol at 0.01 µM. Endosulfan was the only pesticide (among endosulfan, chlordane, toxaphene, and dieldrin) to induce β-gal activity above background; binary mixtures of endosulfan with the other pesticides induced significantly less activity than endosulfan alone.

The test system was considerably less sensitive to endosulfan when mouse ER, rather than human ER, was used to mediate β-gal activity (Ramamoorthy et al. 1997). In similar assays, endosulfan at 10 µM had no effect on β-gal activity in yeast (*Saccharomyces*) transfected with either the human or rainbow trout ER (Andersen et al. 1999). In addition, no effect was observed on transcriptional activation of HeLa cells transfected with plasmids containing an estrogen receptor as a responsive element (Shelby et al. 1996). Endosulfan also did not induce transient reporter gene expression in MCF-7 human breast cancer

cells at an incubation concentration of 2.5 µM (Andersen et al. 1999). Maximum endosulfan-induced ER-mediated luciferase reporter gene expression occurred *in vitro* in a T47D human breast adenocarcinoma cell line at approximately 10 µM, while 50% expression of luciferase occurred at about 5.9 µM; the maximum expression was approximately 59% of the effect from exposure to 0.03 nM estradiol (0.00003 µM) (Legler et al. 1999). Luciferase expression from combined treatment with endosulfan and dieldrin was additive over concentrations ranging from 3 to 8 µM.

Endosulfan at 10 µM induced *in vitro* proliferation of MCF-7 human breast cancer cells to between 2- and 5-fold higher than that seen in hormone-free cells, but appeared to be cytotoxic at approximately 100 µM (Andersen et al. 1999). A similar study showed that endosulfan (technical grade) induced cell proliferation in the MCF-7 human breast cancer cell line at exposure levels of 10 and 50 µM between 2 and 4 times control levels, but not at #2 µM (Wade et al. 1997). Soto et al. (1994, 1995) also demonstrated MCF-7 proliferation at a dose level of 10–25 µM endosulfan, with the maximum cell growth induced by endosulfan achieving 86% of that induced by estradiol, and cytotoxicity occurring at higher exposure levels. In apparent contradiction of these positive findings, endosulfan (isomeric composition not reported) did not substantially affect the growth of either ER-positive (MCF-7) or ER-negative (SK-BR-3) cultured human breast cancer cell lines at concentrations of #35 µM. Endosulfan did severely inhibit cell growth at higher concentrations, and this growth inhibition was synergistic when cultures were incubated with either dieldrin or chlordane (Hsu et al. 1998). In another *in vitro* assay, both α- and β-endosulfan were weakly estrogenic in inducing foci in MCF-7 cultures at 10 µM (but not at lower concentrations), and showed no estrogenic synergism when incubated in combination with dieldrin (Arcaro et al. 1998). In addition to inducing cell proliferation, endosulfan induced proliferation of the progesterone receptor, which is another estrogen-mimicking effect (Soto et al. 1995).

α- and β-Endosulfan each altered the relative quantities of estradiol metabolites *in vitro* in ER-positive MCF-7 human breast cancer cells. The amount of a genotoxic estradiol metabolite, 16α-hydroxyesterone (16α-OHE1), was increased relative to controls and the metabolite 2-hydroxyestrone (2-OHE1), which inhibits breast cell proliferation, was decreased relative to controls (Bradlow et al. 1995), resulting in a slight increase in the 16α-OHE1/2-OHE1 ratio. The authors hypothesized that by producing an increase in the 16α-OHE1/2-OHE1 ratio, endosulfan may increase the risk of estradiol-induced abnormal cell growth in ER-positive tissues such as breast tissue.

The overall evidence indicates that endosulfan administered *in vivo* may be disruptive of reproductive hormone levels in male animals. On the other hand, endosulfan is neither estrogenic nor disruptive of

thyroid or pituitary hormone levels in females *in vivo*, despite its weak estrogenicity in several *in vitro* test systems.

2.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the

child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

The effects of endosulfan have not been studied in children, but they would likely experience the same health effects seen in adults exposed to endosulfan. Data in adults, mostly derived from cases of accidental or intentional acute exposure (ingestion) to large amounts of endosulfan, indicate that the primary target of endosulfan toxicity is the nervous system. The effects are manifested as hyperactivity and convulsions and in some cases have resulted in death (Aleksandrowicz 1979; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Cable and Doherty 1999; Lo et al. 1995; Terziev et al. 1974). These effects have been reproduced in experimental animals.

Results from a few animal studies suggest that, for some end points, young and older animals exhibit different susceptibility. For example, a study conducted in rat pups in which the animals were treated intraperitoneally with 1 mg of technical endosulfan/kg/day for 25 days beginning at 1 day of age found a significant increase in the binding of serotonin to frontal cortical membranes (Zaidi et al. 1985). This increase correlated well with an increase in aggressive behavior. In contrast, exposure of adults to 1 mg/kg for 30 consecutive days did not induce significant changes in the binding or in aggressive behavior (Seth et al. 1986). Without further elaboration, Seth et al. (1986) suggested that the increased sensitivity showed by the pups may be due to the fact that serotonergic receptors develop postnatally. Kiran and Varma (1988) administered endosulfan orally for 4 days at 12.5 mg/kg/day to rats of four different ages (15, 30, 70, and 365 days old) and found that in older animals, endosulfan produced body tremors and muscular contractions, as well as hyperglycemia and reduction in liver glycogen content. None of these effects were observed in the 15-day-old pups, but endosulfan did reduce the activity of

erythrocyte Na^+-K^+-ATPase in this age group. No explanation was offered for this differential effect. If, as discussed in Section 2.4.2, endosulfan-induced convulsive activity is caused by inhibition of GABAergic systems, an immature GABAergic system in the 15-day-old pups may have been responsible for the lack of such activity. The results from these studies suggest that the determination of whether young animals are more susceptible than older ones or vice versa is influenced by the specific neurological response that is affected. The neurological response, in turn, depends on the degree of maturation of the neurotransmitter system(s) responsible for that response.

An additional study reported age-dependent effects. Lakshmana and Raju (1994) found that oral treatment of rat pups with endosulfan from postnatal days 2–10 resulted in changes in the concentration of noradrenalin, dopamine, and serotonin in various brain areas that differed either in magnitude or direction from changes seen in pups treated from postnatal days 2–23. While the results from this study do not necessarily indicate that neonates are more sensitive to the toxic effects of endosulfan, they do show that the duration of exposure in neonates is an important parameter to consider.

Differential susceptibility between young and older animals has also been found regarding other end points. Studies by Sinha et al. (1995, 1997) found that oral treatment of 3-week-old male rats with endosulfan for 90 days resulted in reduced intratesticular spermatid count and increased percent of abnormal sperm at doses lower than those that caused similar effects in 3-month-old rats treated for 70 days. This led the authors to conclude that exposure during a period of testicular maturation when spermatogenesis is in progress may result in disturbed spermatogenesis at sexual maturity.

Results from studies in animals exposed to endosulfan during gestation have provided inconclusive evidence of adverse developmental effects in the offspring. Some effects reported included an increased percentage of resorptions and skeletal variations in the fetuses (Gupta et al. 1978) and decreased fetal weight and length and increased skeletal variations (FMC 1980b). However, the dose levels at which these effects were observed also caused maternal toxicity, suggesting that may have been only secondary to the poor health condition of the mother. Studies in which administration of endosulfan included a period prior to mating as well as through gestation and lactation reported effects such as decrease in mean litter weight also at maternally toxic dose levels (Hoechst 1982). An additional study also reported decreased mean litter weight (Hoechst 1984a), but upon further analysis of the data, the effect was not considered treatment-related (IRIS 2000). Endosulfan was not estrogenic in *in vivo* assays in immature female rats (Raizada et al. 1991; Wade et al. 1997) or mice (Shelby et al. 1996), and exhibited mixed

positive and negative results with respect to estrogenic properties in various *in vitro* assays (see further details in 2.6 Endocrine Disruption).

There is no information regarding pharmacokinetics of endosulfan in children or regarding nutritional factors that may influence absorption of endosulfan. Based on the results from the studies mentioned above, there is no evidence that endosulfan or its metabolites cross the placenta. On the other hand, there is no apparent reason why they would not do so. Endosulfan was detected in the breast milk of rural Khazakhstan women who were environmentally exposed (Lutter et al. 1998). In a study in which lactating goats were administered endosulfan for 28 days, only trace amounts of endosulfan residues were transferred to the nursing kids (Indraningsih et al. 1993). In milk sheep, approximately 1% of radio-activity administered in a single oral dose of ^{14}C-endosulfan was recovered in the milk as endosulfan sulfate at approximately 1 day postdosing; the concentration in the milk declined to very low levels by 8 days postexposure, but was still detectable in milk at 2 ppb at 22 days postexposure (the end of the study) (Gorbach et al. 1968). There is no information on the metabolism of endosulfan in children. Because endosulfan is rapidly eliminated from the body after exposure, there is little likelihood that the chemical from preconception exposures in women would be present in the body during pregnancy or lactation. Although there is evidence that endosulfan induces microsomal cytochrome P-450 in animals (Siddiqui et al. 1987a; Tyagi et al. 1984), the specific mechanism of endosulfan metabolism is not known, and therefore no conclusion about developmental regulation can be drawn. There are no PBPK models for endosulfan.

There are no biomarkers of exposure or effect for endosulfan that have been validated in children or adults exposed as children. Isomers and metabolites of endosulfan were detected in the fat of 30–40% of children hospitalized in agricultural regions of Spain (Olea et al. 1999). The adipose endosulfan was presumably from recent dietary exposure in the light of evidence in a rat study (Dorough et al. 1978), indicating that endosulfan is rapidly eliminated from fat tissues after cessation of dietary exposure. However, methods for obtaining samples of fat are relatively invasive, so adipose endosulfan may not be practicable as a routine biomarker of recent exposure in children. No studies were located regarding interactions of endosulfan with other chemicals in children. Information regarding interactions of endosulfan with other chemicals in humans are limited to anecdotic reports, and inference to what might occur in children based on those reports might be inappropriate.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to endosulfan, reducing body burden, or interfering with the mechanism of action for toxic

effects. Also, no data were located regarding whether methods for reducing toxic effects of endosulfan used in adults might be contraindicated in children. No data were available on whether methods for reducing toxic effects of endosulfan used in adults have been validated in children.

There is no information regarding possible transgenerational effects of endosulfan exposure in humans and the limited data in animals are insufficient to establish whether such effects might occur. For example, a statistically significant increase in chromosomal aberrations was observed in mouse spermatocytes 60 days after initial treatment with oral doses of endosulfan of 6.4 mg/kg/day for 5 days (Usha Rani and Reddy 1986). In male rats, acute exposure to doses of up to 22 mg/kg/day of endosulfan for 5 days did not induce chromosomal aberrations in spermatogonial cells (Dikshith and Datta 1978). The ratios of mitotic index and frequency of chromatid breaks in the two cell types had no correlation with the doses tested and were not significantly different from the control group. Oral administration of 11.6 mg/kg/day of endosulfan to rats for up to 30 days also failed to induce chromosomal damage in spermatogonial cell systems, but it is not known how soon after treatment the animals were killed, and as shown in mouse studies (Usha Rani and Reddy 1986), a latency period of 60 days was required to see chromosomal aberrations in spermatogonia. However, relatively significant changes were observed for mitotic indices (Dikshith et al. 1978).

2.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the

body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to endosulfan are discussed in Section 2.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by endosulfan are discussed in Section 2.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.10 “Populations That Are Unusually Susceptible”.

2.8.1 Biomarkers Used to Identify or Quantify Exposure to Endosulfan

The primary biomarkers for endosulfan exposure include tissue and excreta concentrations of endosulfan, or its metabolite, endosulfan sulfate. Other metabolites that can be detected include endosulfan diol, hydroxyether, and endosulfan lactone (Hayes 1982; WHO 1984). In animals, the metabolites appear in the tissues and excreta following prolonged exposure to endosulfan (Deema et al. 1966; Dorough et al. 1978). These water-soluble metabolites are rapidly formed and excreted in the urine and feces. Elevated levels of both α- and β-endosulfan, but not endosulfan metabolites, were detected in the urine of a pest control worker (who wore protective equipment) after a single 25-minute exposure to endosulfan in a greenhouse application. Urinary endosulfan declined to control levels by about 1.5 days postexposure (Arrebola et al. 1999). α- and β-Endosulfan were detected in the urine of workers who had applied endosulfan on the day prior to urine sampling, and were at lower levels in workers who had been occupationally exposed 1 week prior to sampling (Vidal et al. 1998). Metabolites (endosulfan ether, endosulfan sulfate, and endosulfan lactone) were either infrequently detected or occurred at relatively low levels in the urine. Endosulfan was detected in the serum of an agricultural pilot 30 hours after his

clothes became soaked with endosulfan and methomyl (Cable and Doherty 1999). Endosulfan has been detected in breast milk of rural Khazakhstan women exposed environmentally to endosulfan (Lutter et al. 1998), and endosulfan sulfate has been detected in sheep breast milk following consumption of large oral doses (Gorbach et al. 1968). Isomers and metabolites of endosulfan were detected in the fat of 30–40% of children hospitalized in agricultural regions of Spain, demonstrating that endosulfan accumulates in adipose tissue of young humans after repeated dietary exposure (Olea et al. 1999). No other biomarkers of exposure were identified in the available literature. No studies were located that quantified the concentrations of endosulfan or its metabolites in relation to specific environmental exposure concentrations.

Since endosulfan is a cytochrome P450-dependent monooxygenase inducer, the quantification of specific enzyme activities (e.g., aminopyrine-*N*-demethylase, aniline hydroxylase) may indicate that exposure to endosulfan has occurred (Agarwal et al. 1978). Because numerous chemicals and drugs found at hazardous waste sites and elsewhere also induce hepatic enzymes, these measurements are nonspecific and are not necessarily an indicator solely of endosulfan exposure. However, these enzyme levels can be useful indicators of exposure, together with the detection of endosulfan isomers or the sulfate metabolite in the tissues or excreta.

Evidence of anemia may also be used as a nonspecific biomarker for endosulfan exposure. There is evidence to suggest that endosulfan at levels as low as 1 ppb causes damage to human red blood cell membranes *in vitro* (Daniel et al. 1986) and that it produces other hematologic effects (reduced red blood cells, hemoglobin concentrations, and packed cell volume) in animals following oral exposure to levels ranging from 0.5 to 360 ppm (Das and Garg 1981; Hoechst 1985a; Siddiqui et al. 1987b). However, these effects are not specific enough to be used as biomarkers for exposure to endosulfan *in vivo*.

More recent studies have focused on developing sensitive *in vitro* assays that can serve as biomarkers for chemicals possessing estrogenic activity. Sonnenschein et al. (1995) described a procedure to extract and separate xenoestrogens from ovarian estrogens in human serum followed by determination of their concentration in *in vitro* gene expression assays in MCF-7 breast cancer cells. Such a biomarker seems more discriminative than those discussed above. However, one reservation about the assay, as stated by Sonnenschein et al. (1995), is that estrogen-inducible genes could also be induced by nonestrogenic substances.

2.8.2 Biomarkers Used to Characterize Effects Caused by Endosulfan

Acute clinical signs of neurotoxicity, manifested by hyperexcitability, dyspnea, decreased respiration, tremors, and convulsions, were identified in the available literature as effects caused by high doses of endosulfan. Exposure to high levels of endosulfan in humans may possibly be associated with permanent brain damage as manifested by cognitive and emotional deterioration, memory impairment, and impairment of visual-motor coordination (e.g., inability to perform small tasks) (Aleksandrowicz 1979; Shemesh et al. 1988). The organs most sensitive to longer-term endosulfan exposure appear to be the liver, kidney, and testes. Histopathological and degenerative changes in liver and kidney cells and increased hepatic enzyme activities (aminopyrine, *N*-demethylase, and aniline hydroxylase) have been observed following long-term treatment with low doses of endosulfan. Decay curves for aminopyrine in plasma, which are semiquantitative indices of liver enzyme induction, have been used successfully to demonstrate enzyme induction in pesticide-exposed workers. Decreased red blood cells, hemoglobin, packed cell volume, and IgG, IgM, and γ-globulin levels in the blood have also been detected in animals following exposure to endosulfan (Banerjee and Hussain 1986, 1987; Das and Garg 1981; Hoechst 1985a; Siddiqui et al. 1987b) and thus can be considered as other nonspecific biomarkers of effects. Rats exposed to endosulfan levels as low as 60 ppm were shown to have darker urine and marginal increases in protein and ketone levels in the urine than control animals (Hoechst 1985a). Degenerative effects on reproductive organs and histopathological effects in the kidneys of animals have also been associated with chronic administration of endosulfan. All parameters mentioned are generally nonspecific for endosulfan exposure as numerous other chemicals elicit changes in these end points. See Section 2.2 for other effects caused by endosulfan.

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

2.9 INTERACTIONS WITH OTHER CHEMICALS

The results of two human cases suggest that endosulfan and alcohol might act synergistically to cause death (Demeter et al. 1977). In one death, the blood alcohol concentration was 1.81 g/L (which is not considered lethal), and the quantity of endosulfan consumed (although the exact quantity was not reported) was also unlikely to be fatal when ingested alone. Thus, the authors suggested that the endosulfan and alcohol acted synergistically to result in death. It is also likely that alcohol interfered with

the metabolism of endosulfan in the liver and, therefore, delayed endosulfan elimination. A second fatal outcome was reportedly caused by ingestion of alcohol and Posidor (20% endosulfan and 30% dimethoate in xylene). Dimethoate is an organophosphate insecticide and a potent inhibitor of the cholinesterase; it was considered by the authors to be much more acutely toxic than endosulfan (Demeter et al. 1978). It is likely that the dimethoate and endosulfan acted synergistically. Because of the limitations of these reports, such as multiple chemical exposure and unquantified doses, they provide only suggestive evidence of an interaction.

Endosulfan has been documented to be an enzyme inducer of the cytochrome P450-dependent monooxygenase system in several studies with experimental animals (Agarwal et al. 1978; Den Tonkelaar and Van Esch 1974; Gupta and Gupta 1977a; Kiran and Varma 1988; Siddiqui et al. 1987a; Sriram and Misra 1983). Vitamin A was found to inhibit the activity of cytochrome P450-dependent monooxygenase systems induced by endosulfan. Specific parameters included microsomal protein and cytochrome P-450 contents and the activities of NADPH-cytochrome c reductase, aminopyrine *N*-demethylase, and aniline hydroxylase (Sriram and Misra 1983). Endosulfan and pentobarbital have also demonstrated an interactive effect. Endosulfan reduced the sleeping time induced in male rats by the administration of sodium pentobarbitone (Balasubramamian et al. 1996). The induction of hepatic microsomal enzyme activity and the enhanced metabolism of the pentobarbitone caused by endosulfan are the probable mechanisms, as evidenced by reduced pentobarbitone concentrations in the blood and brain of endosulfan-treated rats (Balasubramamian et al. 1996; Den Tonkelaar and Van Esch 1974; Gupta and Gupta 1977a).

Phenobarbital has a mitigating effect on endosulfan toxicity in rats (Hoechst 1984e). The acute lethal toxicity and neurotoxicity of endosulfan were decreased when phenobarbital (50–70 mg/kg) was given following the appearance of toxic signs. In contrast, diazepam (2–60 mg/kg) delayed death but did not prevent it. It is possible that phenobarbital-induced microsomal enzymes increased the metabolism of endosulfan. In a more recent study, it was found that endosulfan promoted the hypnotic effects of diazepam by prolonging the duration of the loss of righting reflex (Balasubramamian et al. 1996). The authors speculated that endosulfan increased the potency of diazepam by increasing the binding sites for diazepam in the brain synaptic membranes and/or promoted its biotransformation to a longer-acting metabolite, oxazepam (Balasubramamian et al. 1996). In the same study, endosulfan promoted the convulsant action of picrotoxin by shortening the convulsion latency and increasing convulsion frequency. This was thought to have been due to endosulfan increasing the picrotoxin biotransformation

into the active compound (Balasubramamian et al. 1996). It is also possible that endosulfan enhanced the convulsant activity of picrotoxin because both compounds act at the GABA receptor.

Other studies have reported negative findings following the investigation of possible interactions between endosulfan and various compounds. In rats, treatment with endosulfan did not potentiate or aggravate the adverse liver effects induced by pretreatment with carbon tetrachloride (Dikshith and Raizada 1983). The *in vitro* estrogenic effects of endosulfan and dieldrin were found to be additive but not synergistic (Wade et al. 1997). Endosulfan estrogenicity in transfected yeast was inhibited by coexposure with other pesticides. Endosulfan induced human ER-mediated β-galactosidase (β-gal) activity at 100 µM in an estrogen-responsive reporter system in yeast, but did not induce human ER-mediated β-gal activity at #10 µM exposure levels (Ramamoorthy et al. 1997). Binary mixtures of endosulfan with chlordane, toxaphene, and dieldrin induced significantly less activity than endosulfan alone. No additive, antagonistic, or potentiating effects were observed in rats treated with endosulfan and metepa (a chemosterilant used to control insect vectors) (Nath et al. 1978).

Cytotoxic synergism between endosulfan and other organochlorine pesticides was demonstrated in an *in vitro* assay of growth inhibition of ER-negative SK-BR-3 human breast cancer cells, but was not demonstrated in a parallel assay using ER-positive MCF-7 cells (Hsu et al. 1998). The concentration at which 50% growth inhibition (IC_{50}) was achieved in SK-BR-3 cells was approximately 35 µM for endosulfan and dieldrin individually, but the IC_{50} was 0.1 µM for the mixture of the two pesticides. Similarly, the IC_{50} value for chlordane alone was 3.5 µM, but in combination with endosulfan, the IC_{50} was 0.2 µM. A lack of synergism between α- or β-endosulfan and dieldrin was also seen in a foci-induction assay with MCF-7 cells, in which the endosulfan isomers individually were weak inducers of foci at 10 µM (Arcaro et al. 1998).

2.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to endosulfan than will most persons exposed to the same level of endosulfan in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of endosulfan, or compromised function of organs affected by endosulfan. Populations who are at greater risk due to their unusually high exposure to endosulfan are discussed in Section 5.7, Populations With Potentially High Exposures.

The limited toxicity data available for endosulfan suggest that several subgroups of the population may be more susceptible to endosulfan exposure than the general population. These subgroups include the unborn and neonates; the elderly; and people with liver, kidney, or neurological diseases, - effects that have been better characterized in animal studies.

People in the general population with underlying or overt liver or kidney disease, may be at increased risk of adverse health effects following exposure to endosulfan. Evidence from animal studies suggests that endosulfan induces microsomal enzymes and causes histopathological changes in the liver (Dikshith et al. 1984; Gupta and Chandra 1977; Hoechst 1985a). Individuals with liver dysfunction may be more sensitive to the liver toxicity of endosulfan because they cannot detoxify endosulfan as efficiently as individuals with normal liver function. The observation of marked congestion and focal degenerative changes in the kidneys of animals who ingested endosulfan suggests that individuals with renal disease may be more susceptible to the toxic effects of this chemical (Gupta and Chandra 1977; Hack et al. 1995; NCI 1978). Although immunological effects of endosulfan have not been well characterized in animals studies, and altered immunocompetence was reported only in the Banerjee and Hussain (1986, 1987) studies, evidence from studies with other chemicals suggest that immunosupression is a sensitive end point in rodents and other animal species. Therefore, individuals with compromised immune systems such as AIDS patients, infants, and elderly people (who often exhibit a deficiency in immune response because of aging factors) may be more sensitive to endosulfan-induced immunotoxicity than members of the general population (Calabrese 1978). Limited data from animal studies indicate that hematologic effects may result from endosulfan exposure (Das and Garg 1981; Hoechst 1985a; Siddiqui et al. 1987b); therefore, individuals with preexisting anemia or other hematologic disorders may experience intensified systemic toxicity.

The central nervous system is a major target of endosulfan-induced toxicity in both humans and animals (Blanco-Coronado et al. 1992; Boyd and Dobos 1969; Boyd et al. 1970; Garg et al. 1980; Kiran and Varma 1988; Terziev et al. 1974). Therefore, individuals with seizure disorders, such as epilepsy, may be particularly susceptible because exposure to endosulfan may reduce the threshold for tremors, seizures, and other forms of neurotoxicity, as demonstrated in studies in rats (Gilbert and Mack 1995; Gilbert 1992).

Several studies conducted in experimental animals have demonstrated that diets deficient in protein exacerbate the oral toxicity of endosulfan (Boyd 1972; Boyd et al. 1970; Das and Garg 1981). These results suggest that people who consume low-protein diets, such as chronic alcoholics, dieters, food

faddists, various cults, some ethnic groups, the elderly, and some people living in depressed areas or underdeveloped countries, may be more susceptible to the toxic effects of endosulfan.

A detailed discussion of children's susceptibility can be found in Section 2.7, Children's Susceptibility.

2.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to endosulfan. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to endosulfan. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following text provides specific information about treatment following exposures to endosulfan:

Ellenhorn, M.J. 1997. Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning. (2^{nd} ed). Williams and Wilkins, Baltimore. 2047 pp.

2.11.1 Reducing Peak Absorption Following Exposure

Procedures that have been used in an acute exposure situation to limit absorption of endosulfan include the following. In inhalation and dermal exposures, the exposed person is first removed from the source of exposure. Endosulfan rapidly binds to the skin (Hoechst 1986); however, washing the skin thoroughly with mild soap and water may remove any unabsorbed material (Bronstein and Currance 1988; Howland 1990). Since leather absorbs pesticides, it is recommended that leather not be worn in the presence of pesticides and all contaminated leather should be discarded (HSDB 1999). After acute high-dose oral exposures, absorption from the gastrointestinal tract is limited by gastric lavage followed by administration of activated charcoal to adsorb residual endosulfan present in the gut (Blanco-Coronado et al. 1992; Chugh et al. 1998; Howland 1990; Shemesh et al. 1988). Gastric lavage may be indicated in patients who are comatose or at risk of convulsing (HSDB 1999). Oil-based cathartics may facilitate gastrointestinal absorption and, therefore, are not used (Haddad and Winchester 1990; Howland 1990).

2.11.2 Reducing Body Burden

The only relevant information located was that administration of cholestyramine resin may increase fecal excretion of endosulfan trapped in the enterohepatic circulation (Dreisbach and Robertson 1987; Howland 1990; HSDB 1999).

2.11.3 Interfering with the Mechanism of Action for Toxic Effects

The primary life-threatening effect produced following exposure to high levels of endosulfan is respiratory paralysis resulting from the development of seizures. Diazepam (Aleksandrowicz 1979; Blanco-Coronado et al. 1992), midazolam followed by a 30-minute loading dose of phenytoin (Cable and Doherty 1999), and phenobarbitone (Chugh et al. 1998) have been used to control tonic-clonic seizures following massive endosulfan exposures. Lorazepam has also been recommended (HSDB 1999). It is important to control seizures because the anoxia due to convulsions may be the primary cause of severe metabolic acidosis that occurs following acute poisoning (Blanco-Coronado et al. 1992). Fosphenytoin has been suggested if seizures are uncontrollable or recur after diazepam (HSDB 1999). A study using rats showed that phenobarbital is substantially more effective in reducing the neurotoxicity and mortality of endosulfan than is diazepam (Hoechst 1984e). Thus, phenobarbital may be more effective in cases of human exposure as well.

No treatment strategies were located for chronic low-level exposures to endosulfan.

2.12 ADEQUACY OF THE DATABASE

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

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.12.1 Existing Information on Health Effects of Endosulfan

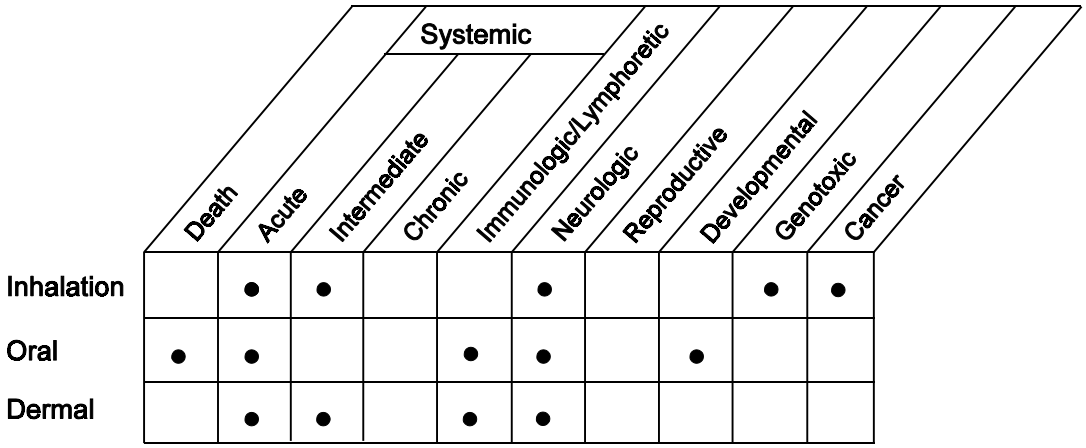
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to endosulfan are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of endosulfan. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Most of the literature reviewed concerning the health effects of endosulfan in humans described case reports of occupational exposure and accidental or intentional ingestion of endosulfan. The cases of occupational exposure to endosulfan concerned exposures of acute-to-intermediate durations, and the cases of oral exposure were exclusively acute-duration exposure situations. The predominant route of exposure in the occupational case reports is believed to be inhalation, but the possibility of some degree of dermal exposure cannot be ruled out. The information on human exposure is limited because the possibility of concurrent exposure to other pesticides or other toxic substances cannot be excluded. In addition, the precise duration and level of exposure to endosulfan generally cannot be quantified from the information presented in these reports.

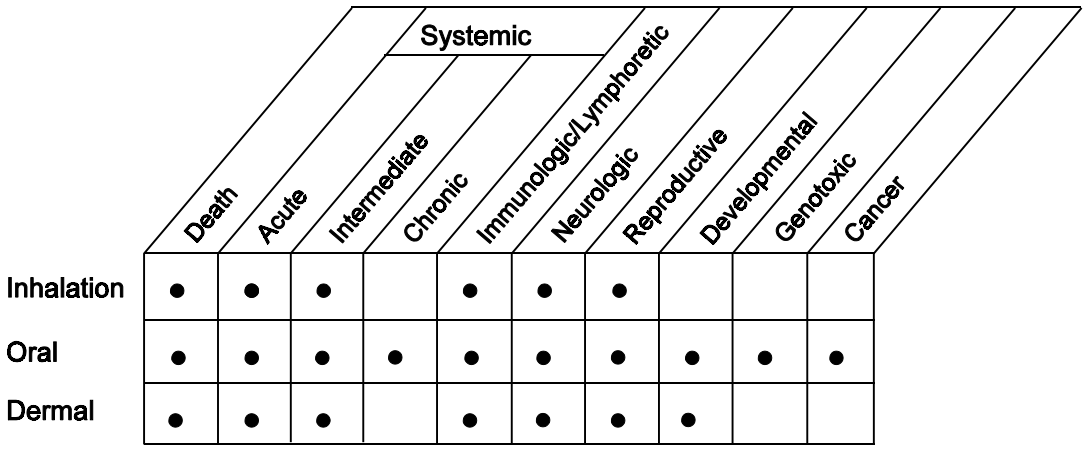
The database for the health effects of endosulfan following ingestion in experimental animals is substantial. However, as can be seen in Figure 2-5, somewhat less information is available on the effects of inhalation and dermal exposure to endosulfan in animals. Furthermore, the health effects associated with acute- and intermediate-duration inhalation and dermal exposure are more fully characterized than those associated with chronic inhalation or dermal exposure. There is no evidence suggesting that the toxicity of endosulfan is route-specific. However, ingested endosulfan should reach the liver sooner.

People living near hazardous waste sites may be exposed to endosulfan primarily via dermal contact with or ingestion of contaminated soils since endosulfan is found bound to soil particles. Another possible

Figure 2-5. Existing Information on Health Effects of Endosulfan



Human



Animal

● Existing Studies

mechanism for oral exposure to endosulfan is the ingestion of pesticide-laden dust from a waste site or treated field carried by the wind and deposited on garden crops. Ingestion of contaminated water is not expected to be a significant route of exposure since endosulfan is not very water soluble and is generally not found in groundwater. Likewise, inhalation exposure to endosulfan via volatilization from contaminated media is not a major route of exposure since endosulfan is not very volatile. For the general population, the primary route of exposure to endosulfan is via ingestion of residues on contaminated foods. Therefore, information on the toxicity of endosulfan following ingestion and dermal exposure is most relevant for individuals living in the vicinity of hazardous waste sites.

2.12.2 Identification of Data Needs

Acute-Duration Exposure. Information is available regarding the effects of acute-duration exposure in humans following inhalation, oral, and dermal exposure to high levels of endosulfan (Aleksandrowicz 1979; Bernardelli and Gennari 1987; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Demeter and Heyndrickx 1978; Ely et al. 1967; Lo et al. 1995; Schuman and Dobson 1985; Shemesh et al. 1988; Singh et al. 1992; Terziev et al. 1974). In animals, information is available following exposures by all three routes (Boyd and Dobos 1969; Boyd et al. 1970; Den Tonkelaar and Van Esch 1974; FMC 1958, 1959a, 1972, 1980a, 1980b; Gilbert and Mack 1995; Gupta and Chandra 1975; Gupta and Gupta 1977a; Gupta et al. 1978, 1981; Hoechst 1966a, 1966b, 1970, 1975, 1983a, 1984e, 1988c, 1989b; Industria Prodotti Chimici 1975; Kiran and Varma 1988; Lakshmana and Raju 1994; Lindquist and Dahm 1957; Misra et al. 1980; Nicholson and Cooper 1977; Siddiqui et al. 1987b; Terziev et al. 1974; Wilson and LeBlanc 1998). Endosulfan may be lethal to humans and animals by all routes of exposure studied, depending on dose (Bernardelli and Gennari 1987; Boereboom et al. 1998; Blanco-Coronado et al. 1992; Boyd and Dobos 1969; Boyd et al. 1970; Demeter and Heyndrickx 1978; FMC 1980a; Gupta and Chandra 1975; Gupta et al. 1978, 1981; Hoechst 1966a, 1966b, 1975, 1983a, 1989b; Lindquist and Dahm 1957; Lo et al. 1995; Nicholson and Cooper 1977; Terziev et al. 1974). The main target of toxicity in humans and animals following acute, high-level exposure by any route is the central nervous system (Aleksandrowicz 1979; Boereboom et al. 1998; Boyd and Dobos 1969; Boyd et al. 1970; Cable and Doherty 1999; Ceron et al. 1995; Chugh et al. 1998; Ely et al. 1967; FMC 1958, 1959a, 1980a, 1981; Gilbert and Mack 1995; Gupta and Chandra 1975; Hoechst 1970, 1975, 1983a, 1984e, 1989b; Kiran and Varma 1988; Nicholson and Cooper 1977; Shemesh et al. 1988; Terziev et al. 1974). Limited information regarding adverse systemic effects has been reported in humans (Blanco-Coronado et al. 1992; Boereboom et al. 1998; Cable and Doherty 1999; Chugh et al. 1998; Demeter and Heyndrickx 1978; Shemesh et al. 1988; Terziev et al. 1974). However, the liver and kidney also appear to be targets of endosulfan toxicity following acute

exposure in experimental animals (with effects on the respiratory and cardiovascular systems that are most likely secondary to central nervous system toxicity) (Boyd et al. 1970; Den Tonkelaar and Van Esch 1974; FMC 1958, 1959a, 1972, 1980a; Gupta and Chandra 1975; Gupta and Gupta 1977a; Hoechst 1970, 1983a, 1989b; Industria Prodotti Chimici 1975; Kiran and Varma 1988; Misra et al. 1980; Siddiqui et al. 1987b; Terziev et al. 1974). No conclusive evidence of developmental toxicity has been presented, mainly because of the questionable quality of the studies available and/or observations of developmental toxicity at maternally toxic doses of endosulfan (FMC 1980b, 1981; Gupta et al. 1978; Hoechst 1982, 1984a).

The data in animals are insufficient to derive an acute inhalation MRL because serious effects were observed at the lowest dose tested (Hoechst 1983a). No acute oral MRL was derived for the same reason. The available toxicokinetic data are not adequate to predict the behavior of endosulfan across routes of exposure. However, the limited toxicity information available does indicate that similar effects are observed (i.e., death, neurotoxicity) in both animals and humans across all routes of exposure, but the concentrations that cause these effects may not be predictable for all routes. Most of the acute effects of endosulfan have been well characterized following exposure via the inhalation, oral, and dermal routes in experimental animals, and additional information on the acute effects of endosulfan does not appear necessary. However, further well conducted developmental studies may clarify whether this chemical causes adverse developmental effects.

Intermediate-Duration Exposure. No information is available on the toxicity of endosulfan to humans following intermediate-duration exposure by the oral route. Only very limited information is available regarding intermediate-duration occupational exposure (Aleksandrowicz 1979). Information is available regarding the effects of intermediate-duration exposure in animals following inhalation, oral, and dermal exposure (Banerjee and Hussain 1986, 1987; Das and Garg 1981; Dikshith et al. 1984, 1988; Garg et al. 1980; Gupta and Chandra 1977; Gupta and Gupta 1977a; Hoechst 1982, 1983b, 1984a, 1984b, 1984c, 1985a, 1985b, 1985c, 1985d, 1987, 1989c; Vos et al. 1982). The targets of toxicity in animals following intermediate exposure by any route appear to be the liver, kidney, reproductive (testes), immune systems, and nervous system (Das and Garg 1981; Dikshith et al. 1984, 1988; Gilbert 1992; Gupta and Chandra 1977; Gupta and Gupta 1977a; Hoechst 1983b, 1984b, 1985a, 1985b, 1985c, 1985d, 1987, 1989c; Lakshmana and Raju 1994; Paul et al. 1995; Sinha et al. 1997; Vos et al. 1982). The data in animals are not sufficient to derive an intermediate-duration inhalation MRL. However, sufficient data were available to derive an intermediate-duration oral MRL. The intermediate-duration oral MRL of 0.005 mg/kg/day was based on immunotoxicity in rats (Banerjee and Hussain 1986). The available

toxicokinetic data are not adequate to predict the behavior of endosulfan across routes of exposure. However, the limited toxicity information available does indicate that similar effects are observed (i.e., hepatic, neurological) in animals across all routes of exposure, but the concentrations that cause these effects may not be possible to predict for all routes. Since the effects of intermediate-duration exposure to endosulfan have been well characterized in experimental animals, additional information on the effects of intermediate-duration exposure to endosulfan is not necessary.

Chronic-Duration Exposure and Cancer. No information is available on the toxicity of endosulfan to humans following chronic-duration exposure by any route. Also no information is available regarding chronic-duration inhalation or dermal exposure in experimental animals. Information is, however, available regarding the effects of chronic-duration exposure in animals following oral exposure (FMC 1959a, 1959b, 1967; Hack et al. 1995; Hoechst 1984b, 1988b, 1989a, 1989c; NCI 1978). The targets of toxicity in animals following chronic oral exposure appear to be the liver and kidney. A chronic-duration oral MRL of 0.002 mg/kg/day was based on increased serum alkaline phosphatase in dogs consuming endosulfan in the diet for 1 year (Hoechst 1989c). Since no data are available for the inhalation route of exposure, a chronic inhalation MRL could not be derived. The available toxicokinetic data are not adequate to predict the behavior of endosulfan across routes of exposure. However, the limited toxicity information available for acute- and intermediate-duration exposures does indicate that similar effects are observed in animals, but the concentrations that cause these effects may not be possible to predict for all routes. Since the effects (or lack thereof) of chronic-duration oral exposure to endosulfan have been well characterized in experimental animals, and since inhalation is not expected to be a major route of exposure for individuals living in the vicinity of hazardous waste sites because of endosulfan's low volatility, additional information on the effects of chronic-duration oral exposure to endosulfan is not necessary. However, a chronic-duration dermal study might be useful to identify more accurately the end points of toxicity and the concentrations at which these effects are observed.

No studies or reports of cancer in humans associated with exposure to endosulfan by any route have been found. The carcinogenicity of endosulfan has been studied in chronic oral bioassays using rats (FMC 1959b; Hack et al. 1995; Hoechst 1989a; NCI 1978) and mice (Hack et al. 1995; Hoechst 1988b; NCI 1968, 1978). The available data in experimental animals were negative or inconclusive. The limited information available on the toxic effects of dermally administered endosulfan suggests that this chemical behaves similarly across both the oral and dermal routes of exposure. However, a study assessing the neoplastic potential of chronic-duration dermal exposure to endosulfan might be valuable.

Genotoxicity. No reliable data on humans exist to indicate whether endosulfan may act by a genotoxic mechanism. The results from available *in vivo* animal studies and *in vitro* studies are mixed, but generally provide evidence that this compound is mutagenic, clastogenic, and induces effects on cell cycle kinetics in two different mammalian species (Dikshith and Datta 1978; Dikshith et al. 1978; Dorough et al. 1978; Dubois et al. 1996; Dzwonkowska and Hubner 1986; Hoechst 1984d, 1988d; Kurinnyi et al. 1982; L'Vova 1984; McGregor et al. 1988; Moriya et al. 1983; Pednekar et al. 1987; Sobti et al. 1983; Usha Rani and Reddy 1986; Usha Rani et al. 1980; Velazquez et al. 1984; Yadav et al. 1982). Some positive results may be suspect, however, because some endosulfan formulations contained epichlorohydrin, a known genotoxic chemical, as a stabilizer. Thus, additional testing verifying the positive results reported by Dikshith and Datta (1978), Dikshith et al. (1978), Dzwonkowska and Hubner (1986), Kurinnyi et al. (1982), L'Vova (1984), Usha Rani and Reddy (1986), Usha Rani et al. (1980), and Velazquez et al. (1984) would be valuable. Also, *in vivo* tests of chromosomal aberrations in exposed human populations would provide valuable information on the genotoxic potential of endosulfan in humans.

Reproductive Toxicity. No information is available on humans to indicate that endosulfan affects reproductive function. Studies have reported that oral endosulfan had no effect on reproductive performance in rats (Dikshith et al. 1984; Hoechst 1982, 1984a). At higher doses than those used in these studies, adverse effects on the testes were observed in male rats that ingested endosulfan, but no assessment of reproductive performance was made (Gupta and Gupta 1977a; NCI 1978). More recent studies that looked at possible effects of endosulfan on spermatogenesis found reduced sperm counts and sperm abnormalities in rats and mice in intermediate-duration studies (Khan and Sinha 1996; Sinha et al. 1995, 1997). Testicular atrophy was observed in rats treated with relatively high doses of endosulfan in the diet for up to 82 weeks (NCI 1978). No information is available on effects on reproductive performance of inhaled or dermally administered endosulfan. Although the available reproductive studies indicate that endosulfan has no adverse effects on reproductive performance in animals following oral exposure, further studies are necessary to clarify the issue, since this apparently contradicts the more recent findings of altered spermatogenesis at comparable dose levels. The oral route of exposure is chosen for further testing because it is most relevant for humans living in the vicinity of hazardous waste sites, adverse testicular effects have been observed following oral exposure. The limited information available on the toxic effects of dermally administered endosulfan suggests that this chemical behaves similarly across both routes of exposure.

Developmental Toxicity. No information is available regarding the effects of endosulfan on human fetal development. No conclusive evidence of developmental toxicity has been presented, mainly because of the questionable quality of the studies available and/or observations of developmental toxicity at maternally toxic doses of endosulfan (FMC 1980b, 1981; Gupta et al. 1978; Hoechst 1982, 1984a). Further testing would be helpful to verify the effects that have been observed and to delineate clearly the doses at which these effects may be expected to occur. Any further testing should be by the oral route of exposure because it is the most relevant for humans living in the vicinity of hazardous waste sites, adverse developmental effects have been observed following oral exposure, and the limited information available on the toxic effects of dermally administered endosulfan suggests that this chemical behaves similarly across both routes of exposure. There is no information about postnatal exposures and any associated postnatal developmental effects.

Immunotoxicity. Limited information is available regarding the effects of endosulfan on the human immune system. However, specially designed studies using rats indicate that both humoral and cellular immune responses are depressed by ingested endosulfan at doses that do not induce any overt signs of toxicity (Banerjee and Hussain 1986, 1987). *In vitro* studies support the possibility that endosulfan affects immune system function (Das et al. 1988). These results demonstrate that immunotoxicity may be a more sensitive end point of endosulfan-induced toxicity than other end points, and humans may be at risk for adverse immune effects following exposure to endosulfan. An intermediate-duration oral MRL was derived based on the observation of depressed immune responses (Banerjee and Hussain 1987). Since the limited information available on the effects of dermally administered endosulfan suggests that this chemical behaves similarly across both routes of exposure and that adverse effects on immune function end points have also been observed *in vitro*, there is no reason to suspect that the immunotoxic effects observed following oral exposure are route-specific. Tests of immunologic function in exposed human populations would provide information as to whether immunosuppression also occurs in humans or whether this effect may be species-specific. Further studies investigating the mechanism of endosulfan-induced immunotoxicity would be helpful since this information may help identify special populations at risk for such effects. In addition, a determination of the threshold dose at which immunotoxic effects occur would be helpful in assessing the risk posed to humans exposed to endosulfan.

Neurotoxicity. Information indicates that the central nervous system is the major target of endosulfan-induced toxicity in humans and animals following acute exposure by any route (Aleksandrowicz 1979; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Boyd and Dobos 1969; Boyd et al. 1970; Cable and Doherty 1999; Chugh et al. 1998; Ely et al. 1967; FMC 1958, 1959a, 1980a; Gilbert and Mack 1995;

Gupta and Chandra 1975; Hoechst 1970, 1975, 1983a, 1984e, 1989b; Kiran and Varma 1988; Lakshmana and Raju 1994; Lo et al. 1995; Nicholson and Cooper 1977; Pradhan et al. 1997; Shemesh et al. 1988; Terziev et al. 1974). The most prominent signs of acute exposure to endosulfan in humans (oral and occupational) and animals (by all routes) are hyperactivity, tremors, decreased respiration, dyspnea, salivation, and tonic-clonic convulsions, which can lead to death. Neurotoxic effects are not always seen following intermediate- or chronic-duration exposure; however, chronic effects were observed in dogs (Hoechst 1989c). Also, two reports indicated that persistent cognitive brain damage may result following acute exposure (Aleksandrowicz 1979; Shemesh et al. 1988). More recent studies in animals have shown changes in neurotransmitter levels and alterations in neurobehavioral processes after exposure to endosulfan (Lakshmana and Raju 1994; Paul et al. 1995). Since the limited information available on the effects of dermally administered endosulfan suggests that this chemical behaves similarly across both routes of exposure, and neurotoxicity has been observed following inhalation exposure as well, there is no reason to suspect that the neurological effects observed following oral exposure are route-specific. Further studies investigating the mechanism for endosulfan-induced neurotoxicity would be helpful since this information might help identify special populations at risk for such effects. Furthermore, although neurotoxic effects have not generally been observed in intermediate- or chronic-duration animal studies, sensitive neurological functional end points (e.g., various reflexes, grip strength, sensory function, motor activity, or nerve conduction velocity), extensive histologic neuropathological evaluations of brain, spinal cord, and peripheral nerves, or evaluations of higher functions such as learning and memory have not been done for long-term exposures to endosulfan. This information would be useful to assess the potential for this chemical to cause permanent neurological damage.

Epidemiological and Human Dosimetry Studies. Most of the literature reviewed concerning the health effects of endosulfan in humans described case reports of occupational exposure or accidental or intentional ingestion of endosulfan (Aleksandrowicz 1979; Bernardelli and Gennari 1987; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Cable and Doherty 1999; Chugh et al. 1998; Demeter and Heyndrickx 1978; Ely et al. 1967; Lo et al. 1995; Shemesh et al. 1988; Terziev et al. 1974). In one case-control study of the relation between occupational exposures to various suspected estrogenic chemicals and the occurrence of breast cancer, the breast cancer odds ratio was not significantly elevated above unity (OR=1.3; 95% CI=0.2–1.2) for occupational exposure to endosulfan compared to unexposed controls (Aschengrau et al. 1998). However, the study was limited by very small sample sizes, and coexposure to other unreported chemicals also occurred. The predominant routes of exposure in the occupational studies are believed to be inhalation and dermal (workers involved in pesticide manufacture, formulation, and application). The information on human exposure is limited because of the possibility of

concurrent exposure to other pesticides or other toxic substances, and the duration and level of exposure to endosulfan generally cannot be quantified from the information presented in these reports. The most likely identifiable subpopulation exposed to endosulfan is pesticide applicators or individuals involved in the production and formulation of endosulfan. Well designed epidemiological studies of these exposed workers, specifically examining the effects of endosulfan on the liver, kidney, reproductive organ of males, and immune system would be useful. Results may show whether these organs and/or systems are adversely affected in humans since they appear to be the major end points of toxicity in experimental animals. Absorption of endosulfan by the subjects of such a study should be confirmed by analysis of blood and urine for endosulfan isomers and endosulfan sulfate. If endosulfan causes adverse effects in any of these target organs or systems, then these end points may be useful tools to monitor endosulfan exposure in individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect.

Exposure. Known biomarkers of exposure to endosulfan include the measurement of endosulfan or its metabolites in tissue and excreta (Deema et al. 1966; Dorough et al. 1978; Gorbach et al. 1968); these measurements can indicate whether absorption of endosulfan has occurred. The presence of the parent compound and its metabolites are specific biomarkers for endosulfan exposure. However, no studies are available that quantify the concentrations of endosulfan or its metabolites in relation to specific environmental exposure levels. Since endosulfan induces cytochrome P450-dependent monooxygenases (Agarwal et al. 1978), the quantification of these specific enzymes may indicate that exposure to endosulfan has occurred. Blood tests, such as decay curves for aminopyrine in plasma, which are semiquantitative indices of liver enzyme induction, have been used successfully in the past to demonstrate enzyme induction in pesticide-exposed workers. Because numerous chemicals found at hazardous waste sites also induce these hepatic enzymes, these measurements are not specific for endosulfan exposure. However, measurements of enzyme activity, together with the detection of the parent compound or its metabolites in tissue or excreta, can be useful indicators of exposure. All of these potential biomarkers require further verification in epidemiological studies. Further studies with focus on the development of methods to separate and measure the estrogenicity of endosulfan in *in vitro* assays would be valuable since these assays are more sensitive and discriminative than other conventional biomarkers. Preliminary results have been presented by Sonnenschein et al. (1995).

Effect. Histopathological and degenerative changes in liver and kidney cells, increased hepatic enzyme activities (aminopyrine *N*-demethylase and aniline hydroxylase), and decreased red blood cells,

hemoglobin, packed cell volume, IgG, IgM, and γ-globulin levels have been detected in animals following exposure to endosulfan and thus may be considered biomarkers of effects (Banerjee and Hussain 1986, 1987; Das and Garg 1981; Hoechst 1985a; Misra et al. 1980; Siddiqui et al. 1987a, 1987b). These parameters can be measured in liver tissue, kidney tissue, and serum. They are generally nonspecific for endosulfan exposure, because numerous other chemicals elicit changes in these end points. All of the endpoints are useful for measuring short- or intermediate-duration exposure, but only kidney effects have been seen following long-term exposure in animals (FMC 1959b; Hack et al. 1995; Hoechst 1989a; NCI 1978). Therefore, there is no need for additional biomarkers of effects for endosulfan, although development of specific, easily measured biomarkers of exposure would be useful to monitor the endosulfan levels in the human population following long-term exposure to endosulfan.

Absorption, Distribution, Metabolism, and Excretion. Indirect evidence describing the occurrence of toxic effects following exposure to endosulfan by all three routes (inhalation, oral, and dermal) indicates that this compound is absorbed by both humans and animals (Bernardelli and Gennari 1987; Boereboom et al. 1998; Blanco-Coronado et al. 1992; Cable and Doherty 1999; Chugh et al. 1998; Deema et al. 1966; Demeter and Heyndrickx 1978; Dorough et al. 1978; Ely et al. 1967; Gorbach et al. 1968; Gupta and Gupta 1979; Nath and Dikshith 1979; Nicholson and Cooper 1977; WHO 1984). No information is available to assess the relative rates and extent of endosulfan absorption following inhalation or oral exposure in humans or animals or dermal exposure in humans. Limited data are available that assess the relative rates and extent of endosulfan absorption following dermal exposure in animals (Hoechst 1986). The data indicate that endosulfan binds to the skin of rats and is only slowly absorbed in the body, with absorption rates decreasing with time. Only about 25% of the bound material was absorbed into the body by 24 hours (Hoechst 1986). Quantitative information that describes the rate and extent of endosulfan absorption following inhalation, oral, and dermal exposure in humans and/or animals would be useful to assess more fully the hazard presented by exposure to endosulfan at various levels from these different routes. In addition, studies investigating the efficiency of endosulfan absorption from dust particles in the gut would be helpful in estimating the hazard posed by this major route of exposure.

Limited information from case reports is available regarding the distribution of endosulfan in humans following oral exposure (Boereboom et al. 1998; Coutselinis et al. 1978; Demeter and Heyndrickx 1978; Demeter et al. 1977). Isomers and metabolites of endosulfan were detected in the fat of 30–40% of children hospitalized in agricultural regions of Spain, demonstrating that endosulfan accumulates in adipose tissue of children after presumably repeated dietary exposure (Olea et al. 1999). Animal studies

describe the distribution of endosulfan following both short-term and long-term oral exposure (Ansari et al. 1984; Braun and Lobb 1976; Dikshith et al. 1984; Gupta 1978; Hoechst 1987; Nath and Dikshith 1979). The available evidence indicates that endosulfan tends to distribute initially to the fatty tissues but accumulates in the kidney with prolonged exposure. No quantitative information is available on the distribution of endosulfan following inhalation exposure, and three studies are available that describe the distribution of endosulfan in animals after dermal exposure (Dikshith et al. 1984; Hoechst 1986; Nicholson and Cooper 1977). Although limited data are available on the rate and extent of endosulfan distribution, the available information indicates the kidney appears to be the organ with the greatest tissue accumulations following both short- and long-term exposure. However, more information on the distribution of endosulfan in humans and animals following exposure to all three routes would be useful in ascertaining whether there are differences across routes of exposure with respect to distribution.

No information is available regarding the metabolism of endosulfan in humans. However, the metabolic pathway of this chemical has been well characterized in several species of experimental animals (Deema et al. 1966; Dorough et al. 1978; Gorbach et al. 1968; WHO 1984). The data indicate that metabolism of endosulfan occurs in both the liver and kidney (Agarwal et al. 1978; Deema et al. 1966; Hoechst 1987; Siddiqui et al. 1987a; Tyagi et al. 1984). Limited data from an acute dermal study showing a dose-related decrease in excretion with increasing dose indicate that the metabolism of endosulfan is saturable (Hoechst 1986).

Information is available regarding excretion of endosulfan and metabolites in humans. Blanco-Coronado et al. (1992) measured total endosulfan in the urine of poisoned individuals shortly after poisoning occurred. However, it could not be ascertained whether the urine was a major or minor excretion route. α-Endosulfan, β-endosulfan, and/or metabolites were present in the urine of humans after intentional oral exposure (Boerebomm et al. 1998) and after occupational exposure either with (Arrebola et al. 1999) or without (Vidal et al. 1998) protective clothing.

No information was located regarding excretion of endosulfan residues in animals following inhalation exposure. Limited data were located regarding excretion in animals following dermal exposure (Hoechst 1986). The routes and extent of endosulfan excretion following oral exposure in animals have been characterized (Deema et al. 1966; Dorough et al. 1978; Gorbach et al. 1968). More data are needed regarding the characterization of the metabolites and the extent of endosulfan excretion following inhalation and dermal exposure in both humans and animals.

Practically all toxicokinetic properties reported were based on results from acute exposure studies. Generally, no information was available regarding intermediate or chronic exposure to endosulfan. Since endosulfan is an enzyme inducer (Siddiqui et al. 1987a; Tyagi et al. 1984), the kinetics of metabolism during chronic exposure probably differ from those seen during acute exposure. Similarly, excretion kinetics may differ with time and dose. Thus, additional studies on the metabolism and excretion of endosulfan during intermediate or chronic exposure would be useful to assess the potential for toxicity following longer-duration exposures. No PBPK models have been developed for endosulfan.

Comparative Toxicokinetics. Most of the reliable data available on the toxicity of endosulfan in humans are from acute exposures where neurotoxicity is the end point of concern (Aleksandrowicz 1979; Ely et al. 1967; Shemesh et al. 1988; Terziev et al. 1974). The same spectrum of effects is seen in animals after acute exposure (Boyd and Dobos 1969; Boyd et al. 1970; Ceron et al. 1995; FMC 1958, 1959a, 1980a; Gilbert and Mack 1995; Gupta and Chandra 1975; Hoechst 1970, 1975, 1983a, 1984e, 1989b; Kiran and Varma 1988; Nicholson and Cooper 1977; Terziev et al. 1974). However, effects on the gastrointestinal tract, liver, kidney, testes, and hematopoietic and immune systems have also been observed in animals (Banerjee and Hussain 1986, 1987; Boyd et al. 1970; Das and Garg 1981; Den Tonkelaar and Van Esch 1974; Dikshith et al. 1984; FMC 1958, 1959a, 1959b, 1980a; Gupta and Chandra 1977; Gupta and Gupta 1977a; Hoechst 1970, 1985a, 1989a, 1989c; Misra et al. 1980; NCI 1978; Siddiqui et al. 1987b; Terziev et al. 1974), and these effects have generally not been observed (or studied) in humans. No toxicokinetic studies have been performed in humans, but there is information on some toxicokinetic aspects of endosulfan in several species of experimental animals (rats, mice, rabbits, and sheep), and there appears to be little difference between the species (Ansari et al. 1984; Braun and Lobb 1976; Dikshith et al. 1984; Deema et al. 1966; Dorough et al. 1978; Gorbach et al. 1968; Gupta 1978; Nath and Dikshith 1979; Nicholson and Cooper 1977; WHO 1984). However, substantial differences exist in the doses required to produce toxicity in male and female rats in acute- (Hoechst 1985c, 1985d, 1990) and intermediate-duration studies (Paul et al. 1995). Differences in the rates of excretion were proposed to account for the differences in sensitivity of male and female rats (Dikshith et al. 1984), but excretion was not directly studied by these authors. Therefore, further studies evaluating the reason for this difference may provide valuable information for estimating acutely toxic doses in humans.

Methods for Reducing Toxic Effects. There is good information on the procedures that may be used to limit absorption of endosulfan following ingestion (Dreisbach and Robertson 1987; Howland 1990). However, limited information exists for removing endosulfan that has bound to the skin

(Bronstein and Currance 1988; Howland 1990). Animal studies indicate that although endosulfan rapidly binds to the skin, its absorption into the bloodstream from the skin is much slower (Hoechst 1986). The slow rate of entry into the bloodstream could provide a good opportunity to limit toxic effects resulting from dermal exposure if a method to remove endosulfan from the skin were found. Earlier data in rats suggested that phenobarbital may be more effective at reducing the acute neurotoxicity and lethality of endosulfan than diazepam (Hoechst 1984e). A more recent 90-day study found that endosulfan increased the potency of diazepam, possibly by increasing the binding sites for diazepam of brain synaptic membranes or by promoting its biotransformation to a longer acting metabolite, oxazepam (Balasubramaniam et al. 1996). Therefore, additional information regarding differences in the effectiveness of these therapies in human exposure situations would also be valuable. In cases of acute intoxication it is critical to control the convulsive activity since this may lead to severe metabolic acidosis and hyperglycemia (Blanco-Coronado et al. 1992). Additional information on the mitigation of the long-term effects of endosulfan (i.e., renal and hepatic toxicity) would also be valuable.

Children's Susceptibility. The information on health effects of endosulfan in humans is derived mainly from cases of accidental or intentional exposure of adults to high amounts of the pesticide, and the main adverse effect is neurotoxicity. No reports of adverse effects in endosulfan-exposed children were found, but it is reasonable to assume that children will exhibit similar signs and symptoms to those in adults under similar exposure conditions. Some studies in animals have provided evidence that young animals respond to endosulfan differently than adult animals (Kiran and Varma 1988; Lakshmana and Raju 1994; Sinha et al. 1995, 1997; Zaidi et al. 1985), but there is no conclusive evidence to suggest that young animals are more susceptible than older ones. Further studies that evaluate a number of different end points in young as well as older organisms would provide valuable information.

No information was located concerning whether the developmental process is altered in humans exposed to endosulfan either prenatally or postnatally. Studies in animals have provided inconclusive evidence (FMC 1980b, 1981; Gupta et al. 1978; Hoechst 1982, 1984a), and further well-conducted research would be helpful to clarify this issue.

No data were located concerning whether pharmacokinetics of endosulfan in children are different from adults. There are no adequate data to determine whether endosulfan or its metabolites can cross the placenta. Studies in animals addressing these issues would provide valuable information. Although endosulfan has been detected in human milk (Lutter et al. 1998), studies in animals showed very little accumulation of endosulfan residues in breast milk (Gorbach et al. 1968; Indraningsih et al. 1993), which

is consistent with the rapid elimination of endosulfan from tissues and subsequent excretion via feces and urine. There are no PBPK models for endosulfan in either adults or children. There is no information to evaluate whether absorption, distribution, metabolism, or excretion of endosulfan in children is different than in adults.

There are no biomarkers of exposure or effect that have been validated in children. There are no data on interactions of endosulfan with other chemicals in children, and the existing data in adults are inadequate to determine whether the same effects will be observed in children. There are no pediatric-specific methods to reduce peak absorption for endosulfan following exposure, or to reduce body burden, or to interfere with the endosulfan's mechanism of action.

Child health data needs relating to exposure are discussed in 5.8.1, Identification of Data Needs: Exposures of Children.

2.12.3 Ongoing Studies

The following ongoing research project was identified in FEDRIP (1999).

Dr. John E. Casida from the University of California Berkeley is investigating the fundamental basis for the selective toxicity of insecticides, including endosulfan, acting at the gamma-aminobutyric acid (GABA) receptor of mammals and insects. The research is sponsored by the National Institute of Environmental Health Sciences.

Dr. G. A. LeBlanc of North Carolina State University is evaluating effects of potentially endocrine-disrupting chemicals, including endosulfan, on steroid hormone biotransformation/elimination processes in daphnids, fish, and mice, and is constructing models of the processes. The work is being funded by the U.S. Department of Agriculture.

Dr. D.E. Woolley from the University of California, Davis, is conducting research aimed at determining the immediate and long-term effects of exposure to environmental toxicants, especially insecticides, in order to obtain basic toxicological data important for determining mechanisms of action in the intact animal, to evaluate the hazard posed by these agents for the health of man and other nontarget mammalian species. The effects of several organochlorine and other insecticides (endosulfan among them) on physiological, neurological, neurochemical and behavioral end points will be determined in rats, as an

example of a mammalian nontarget species. Dr. Woolley's research is sponsored by the U.S. Department of Agriculture.