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2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ENDOSULFAN IN THE UNITED STATES

The manufacture and use of endosulfan as a broad spectrum contact insecticide and acaricide applied to a wide variety of fruits, vegetables, grains, etc. has led to its direct release into the environment. Technical-grade endosulfan contains at least 94% of two pure isomers, α- and β-endosulfan. The α- and β-isomers of endosulfan are present in the ratio of 7:3, respectively. Endosulfan sulfate is a reaction product found in technical endosulfan; it is also found in the environment due to oxidation by biotransformation. Beginning on July 31, 2012, a voluntary cancellation and phase-out of endosulfan began and all uses of this product are scheduled to end by July 31, 2016. The phase-out will be executed in six phases over this 4-year period. During these phases, use of endosulfan on certain types of crops and products are scheduled to end.

After its release to the environment, endosulfan undergoes a variety of transformation and transport processes. In soil, endosulfan sulfate is the major degradation product from the biodegradation of endosulfan and is considered to be more persistent than the parent compound. Neither endosulfan nor its biodegradation products are expected to be mobile in soil. In an aerobic soil metabolism study using five different soils, half-lives of α-endosulfan ranged from 35 to 67 days and half-lives of β-endosulfan ranged from 104 to 265 days with endosulfan sulfate as the major metabolite. Soil erosion, run-off, spray drift and atmospheric deposition contribute to releases of endosulfan to aquatic ecosystems. Endosulfan transported to water is expected to eventually partition to sediment. In water, endosulfan is hydrolyzed to the less toxic endosulfan diol with a half-life of approximately 1 month at pH 7. Endosulfan has a relatively high potential to bioaccumulate in fish and other aquatic organisms. Volatilization from soil, water, or plant surfaces may occur over time for endosulfan.

Due to its potential for long-range transport, endosulfan is detected in air at both source and non-source locations. Remote Arctic air concentrations range from 3.3 to 8.3 pg/m^3. Rural source areas where endosulfan may be used tend to have higher reported concentrations (18–82 pg/m^3), with spikes reported during growing seasons. Data obtained from the U.S. Geological Survey (USGS) National-Water Quality Assessment (NAWQA) Program suggest that endosulfan generally has low rates of detection in groundwater. Surface water concentrations are highly variable, but are generally highest in water bodies that drain areas of high agricultural use.

Endosulfan and endosulfan sulfate have been detected in a variety of food items. Typical values are <0.1 ppb; however, maximum values for some food items were nearly 100 ppb. It has also been detected in fish and seafood at varying levels. The general population is primarily exposed to endosulfan through dietary intake. Pesticide applicators and crop pickers may be exposed to higher levels via inhalation, accidental ingestion, and dermal exposure as compared to the general population.

2.2 SUMMARY OF HEALTH EFFECTS

The main target of endosulfan in humans and animals is the nervous system. Exposure to high amounts of endosulfan by any route produces over-stimulation of the central nervous system resulting in hyperactivity, tremors, decreased respiration, dyspnea, salivation, tonic-clonic convulsions, and eventually death. Endosulfan does so by antagonizing gamma-aminobutyric acid (GABA) function, an inhibitory neurotransmitter system, thus allowing stimulary activity to manifest itself unopposed. Other effects such as respiratory, gastrointestinal, cardiovascular, renal, and metabolic effects may be secondary to a prolonged status epilepticus. The latter is defined clinically as seizures lasting for more than 5 minutes, or two or more discrete seizures without recovery of consciousness in between. Refractory status epilepticus was the most common cause of death among 52 patients admitted to an emergency facility. In that report, the amount of endosulfan ingested being >35 g was the independent variable that best predicted patient mortality. Very few studies provided information that allowed estimation of a dose. In one study, a dose of 260 mg endosulfan/kg caused the death of a 43-year-old man. In another study, a dose of approximately 2,571 mg endosulfan/kg was lethal to a 36-year-old man. According to one study, 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. There are two cases of possible permanent neurological impairment following acute exposure to high amounts of endosulfan. Follow-up of additional cases of accidental or intentional exposure to endosulfan is necessary to confirm the results of these two studies.

A few studies of environmentally exposed humans have provided mixed results regarding associations between endosulfan and health effects. In these studies, endosulfan was one of many organochlorine compounds or pesticides evaluated. A case-control study of individuals from India diagnosed with Alzheimer's disease found no significant association between levels of α- or β-endosulfan in blood and

risk of the disease. A cross-sectional study of people from Brazil found that serum levels of triiodothyronine (T3) were associated with lower serum concentrations of β-endosulfan in men and with higher β-endosulfan concentrations in women (associations also existed for other pesticides). No significant associations were seen between serum levels of endosulfan and serum free thyroxine (T4) or thyroid-stimulating hormone (TSH). See below for studies that evaluated reproductive and developmental effects.

Studies of endosulfan induces the same type of effects in animals after acute oral doses ≥1.8 mg/kg. Most animals studies have been conducted with technical endosulfan, but a few have examined the effects of the pure α- and β-isomers. Cerebral congestion and edema are often seen in animals that died following acute ingestion of endosulfan. Hyperactivity has also been reported in animals following inhalation and dermal exposure. Mice generally appear to be more sensitive to the lethal effects of endosulfan than rats. Female rats are more sensitive to the lethal effects of endosulfan than male rats. Tonic contractions of the muscles of the extremities, face, and jaw were reported in dogs in a 12-month study. In general, long-term studies have not reported compound-related morphological alterations in tissues of the nervous system. Repeated exposure of immature rats to doses in the range of 2–6 mg endosulfan/kg/day by gavage has also induced changes in the levels of neurotransmitters in the brain and alterations in neurobehavioral tests. However, repeated exposure of adult rats to doses near 30 mg endosulfan/kg/day via the diet in one study and near 46 mg/kg/day in another study did not significantly affect the results of a functional observational battery (FOB) that included examination of autonomic function, posture and gait, and behavior. In rats, endosulfan was also shown to lower the threshold to seizures caused by a subsequent challenge dose of endosulfan or by electrical stimulation of a certain area of the brain.

Studies in animals, mostly by the oral route, have described a wide range of systemic effects of endosulfan including respiratory, gastrointestinal, hematological, hepatic, renal, endocrine, and metabolic effects; alterations in body weight have also been reported. Many of these effects are secondary to severe neurological effects, particularly in acute-duration studies that used relatively high doses of endosulfan. Acute lethal or near-lethal doses have resulted in congestion in the lungs, liver, kidneys, stomach, and intestines. Intermediate-duration studies have reported serious effects such as myocardial fiber degeneration, and degenerative changes in the liver and kidneys in Wistar rats dosed with 1 mg/kg/day technical endosulfan. The same dose level induced degenerative changes in the endocrine pancreas from New Zealand white rabbits. A higher dose of 2 mg/kg/day induced myocardial fiber edema and pancreas histopathology in Wistar rats. None of these studies provided information on clinical signs. In addition, in all of these studies, endosulfan was administered by gavage and, since only one dose level was tested,

no-observed-adverse-effect levels (NOAELs) were not defined. In contrast, a 90-day dietary study in Wistar rats reported that doses up to 37.2 mg/kg/day of technical endosulfan in males and 45.5 mg/kg/day in females did not induce significant compound-related histological alterations in the liver and kidneys; the heart and pancreas were not examined. This suggests that the mode of administration of endosulfan (i.e., diet versus gavage), plays an important role in the toxicity of the chemical, dietary being the more relevant for exposure of the general population. Chronic-duration studies with endosulfan have reported few systemic effects. Increased incidence of glomerulonephrosis and aneurysms of the blood vessels were reported in Wistar rats exposed for 2 years to doses of 3–4 mg/kg/day technical endosulfan; the NOAELs were 0.6–0.7 mg/kg/day and lowest-observed-adverse-effect levels (LOAELs) were 2.9 mg/kg/day for males and 3.8 mg/kg/day for females. Studies in mice exposed to up to 3 mg/kg/day technical endosulfan via the diet reported no toxicity assessed by monitoring clinical signs, hematology tests, and organ histopathology. A 1-year dietary study in dogs reported abdominal and jaw muscle spasms at the highest dose tested, approximately 2 mg/kg/day technical endosulfan. This effect was likely caused by hyperactivity of the nerve fibers innervating the muscles rather than a direct effect of endosulfan on the muscles. Application of endosulfan onto the skin of animals in doses that induced neurological signs also induced a spectrum of systemic effects similar to that observed following oral exposure to endosulfan. Nose-only exposure of rats to concentrations of aerosolized endosulfan $\geq$3.6 mg/m^3 for 4 hours induced frank neurological effects. Repeated exposure to up to 2 mg/m^3 6 hours/day, 5 days/week for a total of 21 out of 29 days did not result in significant alterations in gross or microscopic appearance of tissues and organ of rats.

Endosulfan was not a skin sensitizer when tested in a group of farm workers. Since no studies have been conducted of populations with prolonged exposure to low levels of endosulfan, it is unknown whether such exposure could alter immunocompetence. Studies of subjects who were acutely exposed to high amounts of endosulfan intentionally or accidentally by the inhalation, oral, or dermal routes have not provided information regarding immunological end points. Intermediate- and chronic-duration studies that conducted microscopic examination of lymphoreticular tissues of rats, mice, and dogs did no show significant alterations. Maximal doses were up to 7.3 mg/kg/day in mice, 48 mg/kg/day in rats, and 1.8 mg/kg/day in dogs. Endosulfan suppressed both cell-mediated and humoral immune responses in male Wistar rats in intermediate-duration studies; the NOAEL and LOAEL values were 0.45 and 0.9 mg/kg/day, respectively. Since these are the only studies that tested immunocompetence, it would be helpful to try to replicate the results. However, the immune system is known to be a sensitive target for organochlorine compounds.

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It is unknown whether endosulfan is a reproductive toxicant in humans. A study of men from Turkey could not demonstrate conclusively that environmental exposure to endosulfan, assessed by measuring endosulfan in adipose tissue, was associated with fertility in men. A study of men and women from Brazil reported a significant negative association between serum levels of luteinizing hormone (LH) in peri- and postmenopausal women and serum levels of α- and β-endosulfan (as well as for a number of other pesticides). A significant negative association also was apparent for serum levels of follicle-stimulating hormone (FSH) and α-endosulfan in the same groups of women. No significant association was found between levels of pesticides and sex hormone levels in premenopausal women or between endosulfan and levels of testosterone in men. There was no indication that there had been fertility problems or other health concerns among the men and women participants.

Endosulfan induced adverse reproductive effects in animals, but results from some studies are difficult to reconcile. For example, doses of 6 mg/kg/day administered to Sprague-Dawley rats on gestation days (GDs) 6–19 did not affect post-implantation loss, but doses of 1 mg/kg/day given to Wistar rats on GDs 6–20 significantly increased post-implantation loss. Differences in strain sensitivity may have played a role. The lowest LOAEL for a reproductive end point was 0.8 mg/kg/day for histological alterations in the testes and alterations in sperm parameters in 3-week gavage studies in mice. Intermediate-duration gavage studies also reported alterations in sperm parameters in rats dosed with 2.5–5 mg/kg/day endosulfan. No sperm alterations were reported in rats dosed for 8 weeks with 2.9 mg/kg/day endosulfan via the diet. Multi-generation reproductive studies in rats did not find alterations in fertility or fecundity. This may be due to the fact that rats produce and ejaculate 10 times more sperm than are necessary for normal fertility and litter size. Gavage doses of ≥7.5 mg/kg/day endosulfan for 15–30 days reduced serum testosterone, LH, and FSH in male rats; dietary doses of 2.9 mg/kg/day for 8 weeks did not. Serum testosterone also was reduced in male rats administered 5 mg/kg/day technical endosulfan for 10 days or 30 days, in male mice administered 0.8 mg/kg/day technical endosulfan for 3 weeks, and in male rabbits dosed with 1 mg/kg/day technical endosulfan for 60 days. Except for a report of testicular necrosis and aspermatogenesis in male rats dosed with 48 mg/kg/day endosulfan via the food, long-term studies with endosulfan have not reported morphological alterations in the reproductive organs of rats, mice, or dogs. The NOAEL in the NCI study was 20 mg/kg/day. Repeated inhalation or dermal exposure of rats to endosulfan also did not induce morphological alterations in the reproductive organs. Endosulfan was neither estrogenic nor anti-estrogenic when administered orally to rats or mice; studies *in vitro* have provided mixed results.

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A limited number of studies in humans have provided suggestive evidence of associations between maternal exposure to endosulfan and developmental alterations in the offspring including autism spectrum disorders, alterations in thyroid function, neural tube defects, and delayed sexual maturity in male children. After considering the strengths and limitations of the individual studies, no definite conclusions could be drawn. A recent study of Chinese women found no significant association between levels of α-endosulfan in maternal or cord blood and infant birth weight.

Endosulfan induced adverse developmental effects in animals; most studies were conducted in rats. It should be noted that since not all studies provide information regarding maternal effects, it is not totally clear whether the developmental effects occur only in the presence of maternal toxicity. Perinatal exposure of rats to endosulfan induced skeletal variations, reduced offspring weight, and altered sperm parameters in adult male offspring that were not directly exposed, but received gestational and/or lactational exposure to endosulfan. Three recent studies in which offspring from rats and mice also were not directly exposed, but received gestational and/or lactational exposure to endosulfan (0.61–1 mg/kg/day) reported changes in levels of neurotransmitters (GABA, glutamate, dopamine, norepinephrine, serotonin) in the brain of the adult offspring. Two studies in which Wistar rats were exposed to 1 mg/kg/day endosulfan (only dose level tested) by gavage on GDs 6–20 reported significant increases in the incidences of gross, visceral, and skeletal anomalies, and hepatocyte and renal tubule epithelium degeneration in GD 20 fetuses. The lowest developmental LOAEL was 0.61 mg/kg/day for neurochemical alterations in the brain from adult rats born to dams exposed by gavage during gestation and lactation and for an 11% reduction in body weight in Sprague-Dawley male rat pups on postnatal day (PND) 21 after treatment of the dams also by gavage during the entire gestation and lactation periods; the 0.61 mg/kg/day dose was the lowest dose tested in these studies. Interestingly, treatment of Wistar rats with a much higher dose of endosulfan of 29.8 mg/kg/day via the diet on GDs 6–21 and during lactation resulted in a reduction of 11% and 4% in male and female pup weight, respectively, on PND 21. The apparent difference in sensitivity may be related to the different strains used and to the different mode of administration of the test material (i.e., gavage versus the diet).

It should be mentioned that a recent study reported maternal and developmental effects in mice at doses of endosulfan considerably lower than other studies. In that study, pregnant C57BL/6J mice were fed a diet containing 0.03 ppm (30 µg endosulfan/kg food) technical endosulfan during gestation and lactation (approximately 0.006 mg endosulfan/kg/day using a default food consumption value of 0.0048 kg/food/day and body weight of 0.025 kg for the mice). Dams and pups were sacrificed at weaning, and the liver and kidneys were examined microscopically. The pups' livers showed some

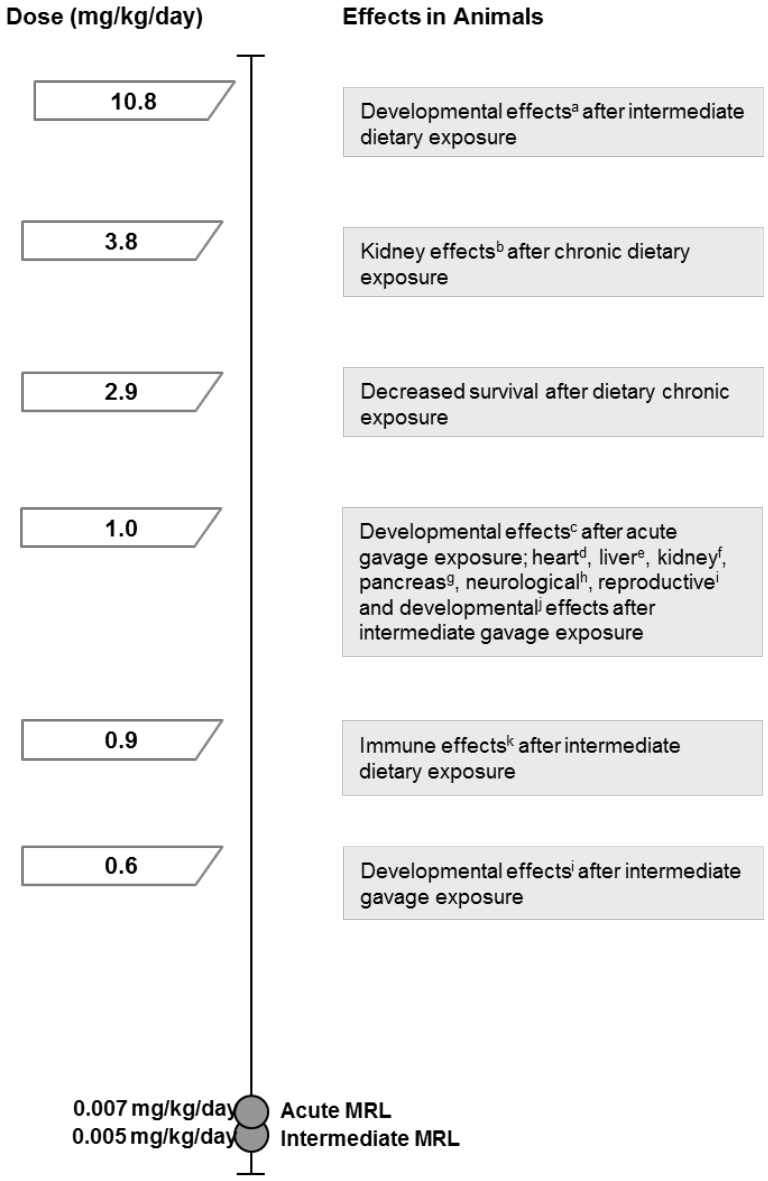
congested sinusoids and necrotic hepatocytes. In the kidneys, some tubules showed epithelium detachment or degeneration and interstitial hemorrhage. Similar effects were reported in dams, including alterations of the hepatic lobules associated with hydropic degeneration, fatty change, and foci of necrotic cells. Maternal kidneys showed atrophy of glomeruli and detachment of the epithelium of the renal tubules and interstitial hemorrhage. While no other study has examined the liver or kidneys of pups from mice exposed to endosulfan during gestation and/or lactation, intermediate- and chronic-duration studies have not reported histological alterations in the livers or kidneys from mice administered endosulfan via the diet in doses up to 3 orders of magnitude higher than those in this study. It would be helpful to try to replicate the findings of this study.

Very limited information is available regarding humans exposed to endosulfan and cancer. A case-control study of women exposed to estrogenic substances at work found no association between exposure to endosulfan and other chemicals and breast cancer based on three cases among exposed and seven cases among controls. A small study of children in India presented suggestive evidence of increased incidence of hematological malignancies among children living near areas sprayed with endosulfan. However, the small sample size (n=26) and little control for potential confounders preclude drawing firm conclusions.

The carcinogenicity of endosulfan has been studied in chronic oral bioassays using rats and mice. While some of these studies 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 better quality studies showed 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. 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, but some found evidence of promotion activity. The EPA has not classified endosulfan as to its carcinogenicity.

Heath effects of endosulfan ingestion in laboratory animals and the dose ranges at which these effects occur are shown in Figure 2-1. An estimate of an oral dose posing minimal risk to humans (MRL) is also presented in this figure. An MRL is an estimate of the daily human exposure that is likely to be safe over a certain period of exposure. MRLs are not intended to define clean-up or action levels, but are intended only to serve as a screening tool to help public health professionals decide where to look more closely. Therefore, MRLs are set at levels well below where effects have been observed.

Figure 2-1. Health Effects of Ingesting Endosulfan



[a]Decreased pup's body weight
[b]Glomerulonephrosis
[c]Decreased sperm count in offspring
[d]Myocardial fiber degeneration
[e]Vacuolar degeneration
[f]Tubule cell degeneration
[g]Degenerative changes
[h]Hyperexcitability and brain hemorrhage
[i]Post-implantation loss and uterus alterations
[j]Visceral and skeletal anomalies; liver and kidneys degeneration in fetuses
[k]Decreased immunocompetence
[l]Reduced pup's weight

2.3 MINIMAL RISK LEVELS (MRLs)

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

Inhalation MRLs

No inhalation MRLs were derived for endosulfan due to inadequacies of the database. Information regarding inhalation exposure to endosulfan by humans was inadequate for derivation of inhalation MRLs (Aleksandrowicz 1979; Aschengrau et al. 1998; Ely et al. 1967; Rau et al. 2012; Roberts et al. 2007). 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. Of these reports, only in the cases reported by Aleksandrowics (1979) and Ely et al. (1967) was there evidence of exposure solely to endosulfan. Aschengrau et al. (1998) did not find a significant association between women exposed to estrogenic substances at work (endosulfan was one of them) and breast cancer. Rau et al. (2012) provided suggestive evidence of increased incidence of hematological malignancies among children residing near areas sprayed with endosulfan. The results of Roberts et al.

(2007) suggested that maternal exposure to endosulfan during key periods of gestation might be associated with the development of autism spectrum disorders.

The acute-duration (1–14 days) inhalation data available in animals are limited to a single LC_{50} study. In that study, male and female Wistar rats (5/sex/group) were exposed nose-only to various concentrations of aerosolized technical endosulfan for 4 hours and observed for 14 days. Trembling and ataxia were observed in rats exposed to the lowest concentrations tested, 12.3 mg/m^3 for males and 3.6 mg/m^3 for females (Hoechst 1983a). Necropsy of the animals that died during the study showed sporadic dark red foci the size of a pinhead in the lungs. Autopsy of animals killed at the end of the study did not reveal macroscopic abnormalities. This study is considered inadequate for derivation of an acute-duration inhalation MRL for endosulfan since the lowest concentration tested was a serious LOAEL and a NOAEL could not be defined.

Only one intermediate-duration (15–364 days) inhalation study was located in the available literature. In that study, groups of male and female Wistar rats (15/sex/group) were exposed nose-only to concentrations of 0, 0.5, 1, and 2 mg/m^3 aerosolized technical endosulfan 6 hours/day, 5 days/week for a total of 21 exposures over a 29-day period (Hoechst 1984c). Ten rats per sex per group were killed 1 day after the last exposure and the remaining 5 rats/group were killed 29 days later. At termination, the rats were subjected to a complete necropsy and the major organs were weighed. Blood samples were collected for hematology and clinical chemistry tests. A comprehensive number of tissues and organs were processed for microscopic examination. There were no significant alterations in behavior or general health with the exception of one male rat from the high concentration group that was in poor condition from day 12 of the study. There was no mortality during the exposure period or the recovery period. The body weight of the high-concentration male group was lower than the control group from day 20 of the exposure period (<10% difference with controls of day 20). This coincided with a marked decrease in food consumption in that group at that time. While the mean weight of this group remained below controls for the remainder of the study, the rate of growth did not appear to be affected. Results from hematology and clinical chemistry tests showed occasional significant differences between exposed and control rats, but values were still within normal limits for the strain of rat, and in many cases, there was no dose-response relationship. Examination of organs and tissues did not show compound-related gross or microscopic alterations. Because no adverse effects were reported in this study, it is not a suitable basis for an MRL.

No chronic-duration (≥365 days) inhalation MRL was derived for endosulfan due to lack of studies.

Oral MRLs

- An MRL of 0.007 mg/kg/day has been derived for acute-duration (1–14 days) oral exposure to endosulfan based on adverse neurological signs in rabbits.

Acute-duration oral data in humans are available from cases of accidental or intentional ingestion of endosulfan. Some of these cases resulted in death (Bernardelli and Gennari 1987; Blanco-Coronado et al. 1992; Boereboom et al. 1998; Eyer et al. 2004; Lo et al. 1995; Moon and Chun 2009; Parbhu et al. 2009; Terziev et al. 1974). Although doses were estimated in some of these reports, they cannot be used for MRL derivation because ATSDR does not base MRLs on serious effects such as seizures or lethal doses.

The animal database provides information mainly on lethal doses of endosulfan and neurological effects. Systemic effects such as organ congestion were also reported at doses that induced neurological effects. The lowest LOAEL in an acute-duration oral study was 1 mg/kg/day for a 53% decrease in sperm count in male offspring of Druckrey rats that were dosed by gavage on GDs 12–21 (Sinha et al. 2001); since only one dose level was tested, the true LOAEL may be lower. Moreover, no information was provided in this study regarding clinical signs in the dams during treatment, other than no mortality occurred. A relatively low LOAEL was reported for a 28% reduction in sperm count and reduced testes weight in guinea pigs dosed by gavage with 2.5 mg/kg/day technical endosulfan for 14 days, this was the lowest dose tested (Umar et al. 2012). The lowest LOAEL for neurological effects was reported in a gestational exposure study in pregnant female New Zealand rabbits that showed hyperactivity and convulsions within 4 days after administration of daily gavage doses of 1.8 mg/kg/day (MacKenzie et al. 1981); the NOAEL was 0.7 mg/kg/day. In a study by Bury (1997), increased incidence of neurological signs was also reported in female Wistar rats within 8 hours after gavage administration of a single dose of 3 mg/kg of endosulfan; the NOAEL was 1.5 mg/kg. Of these studies, the study by Sinha et al. (2001) is not appropriate for MRL derivation since only one dose level was tested and there was no information regarding maternal effects. In the Umar et al. (2012) study, the lowest dose tested was a LOAEL; thus, as in the Sinha et al. (2001) study, the true LOAEL may be lower. Therefore, the Bury study (1997) and Mackenzie et al. (1981) study will be considered for potential derivation of an acute-duration oral MRL for endosulfan.

In the Bury study (1997), male and female Wistar rats (10/sex/treated group, 20 controls/sex) were administered a single dose of technical endosulfan (0, 6.25, 12.5, 25, 50, or 100 mg/kg for males; 0, 0.75, 1.5, 3, 6, or 12 mg/kg for females) by gavage in 2% (v/v) mucilage in deionized water. Rats were

observed for 15 days, during which time they were administered a functional observational battery (FOB) that assessed multiple parameters and were tested for motor activity. The rats were sacrificed 3 weeks after dosing, and multiple levels of the central and peripheral nervous system were examined microscopically. Neurological signs developed in 10/10 males at ≥25 mg/kg and in 10/10 females at ≥3 mg/kg within 8 hours after dosing. Females dosed with 3 mg/kg endosulfan showed increased incidence of squatting posture, stilted gait, straddled hindlimbs, decreased spontaneous activity, bristle coat, and irregular respiration and panting. The incidences of various signs varied, but all 10 female rats dosed with 3 mg/kg showed stilted gait and squatting posture (100% incidence). No clinical signs were noted in subsequent days. There were no compound-related alterations in body weight or in the FOB and motor activity tests, or in the pathological evaluations at termination. Based on the increased incidence of clinical signs seen in female rats, LOAEL and NOAEL values of 3 and 1.5 mg/kg, respectively, can be defined in the study.

In the MacKenzie et al. (1981) study, mated female New Zealand White rabbits (20/dose group) were administered technical endosulfan by gavage in corn oil in doses of 0, 0.3, 0.7, or 1.8 mg/kg/day from GD 6 to 28; dams were sacrificed on GD 29. Body weight and clinical signs were monitored throughout the study. Reproductive and developmental parameters were evaluated at termination. Since deaths occurred in the high-dose group (not totally clear when), 6 mated females were added to this group for a total of 26 dams. Neurological signs were observed in three high-dose dams within 4 days of the start of treatment (in one female on Dd 6, the day of the first dose, and in two females on GD 10, after four doses). The signs consisted of noisy and rapid breathing, hyperactivity, and convulsions. No such signs occurred in the other treated groups or in the control group. Although the incidence of neurological effects of 3/26 is not statistically different from 0/20 in the other groups ($p=0.1713$, Fisher Exact Test), it is appropriate to consider the 1.8 mg/kg/day dose level a LOAEL based on the biological significance of the effect. Neurological effects are characteristic of endosulfan and other chlorinated pesticides in humans and animals. Moreover, an additional study in rabbits reported clinical signs including hyperexcitability, dyspnea, hyperpnea, intermittent intervals of tremors and tonic-clonic convulsions, thrashing against the cage walls, depression, and forelimb extension leading to death in 1/9 and 2/9 New Zealand White male rabbits 10–40 minutes following gavage dosing with 1.5 or 3 mg/kg endosulfan, respectively (Hatipoglu et al. 2008). Therefore, the dose level of 1.8 mg/kg/day in the MacKenzie et al. (1981) study is considered an acute LOAEL for neurological signs; the NOAEL is 0.7 mg/kg/day.

Incidence data for neurological signs from the Bury study (1997) and MacKenzie et al. (1981) study were considered for MRL derivation using the benchmark dose (BMD) approach. Inspection of the data from

the Bury study (1997) shows that two clinical signs appeared to be the most sensitive, stilted gait and squatting posture, both occurring with a 100% incidence (10/10, all responders) in female rats that exhibited clinical signs at 3 mg/kg. Since neither control rats nor rats dosed with 0.75 or 1.5 mg/kg endosulfan exhibited such signs (0/10, no responders), these data are not considered adequate for BMD analysis (EPA 2012). Still, the data are adequate to define NOAEL and LOAEL values for clinical signs of 1.5 and 3 mg/kg endosulfan, respectively.

Incidence data for neurological signs in rabbits occurring within 14 days after dosing started in the MacKenzie et al. (1981) study were analyzed using the BMD approach. The incidence data were 0/20, 0/20, 0/20, and 3/26 in the control, 0.3, 0.7, and 1.8 mg/kg/day dose groups, respectively. Models in the EPA Benchmark Dose Software (BMDS version 2.1.1) were fit to the data set to determine potential points of departure (PODs) for the MRL. Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all of the models providing adequate fit to the data, the lowest benchmark dose (BMDL, the lower limit of a one-sided 95% confidence interval on the BMD) is selected as the POD when differences between the BMDLs estimated from these models are >3-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) is chosen. In accordance with EPA (2012f) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated for all models. For continuous data such as changes in body weight, in the absence of a clear criteria as to what level of change in body/organ weight or body weight gain should be considered adverse, the BMR is defined as a change in weight or weight/gain equal to 1 standard deviation from the control mean (EPA 2012f). Using the criteria for model selection mentioned above, the Gamma model (BMD_{10} 1.76 mg/kg/day; $BMDL_{10}$ 1.23 mg/kg/day) was selected as the best model to fit the incidence of clinical signs in pregnant female rabbits. However, the $BMDL_{10}$ of 1.23 mg/kg/day is not only very close to the BMD_{10} of 1.76 mg/kg/day, a dose that caused serious effects in the study, but it is even closer to a dose of 1.5 mg/kg/day, which caused the same type of serious clinical signs and even death in one of nine rabbits in the Hatipoglu et al. (2008) study, as mentioned above. Taking this into consideration and in the interest of protecting human health, the NOAEL of 0.7 mg/kg/day for clinical signs in the MacKenzie et al. (1981) study is preferred as the POD for derivation of an acute-duration oral MRL for endosulfan. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the NOAEL of 0.7 mg/kg/day results in an acute-duration oral MRL of 0.007 mg/kg/day for endosulfan. A detailed description of the MacKenzie et al. (1981) study is presented in Appendix A.

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

No relevant oral data in humans were located. Although there seems to be a considerable number of intermediate-duration oral studies in animals providing information on systemic effects and also immunological, neurological, reproductive, and developmental effects, many suffer from limitations including inadequate reporting of the results or only one dose level was used. An unequivocal target for endosulfan was not identified in the available studies. The lowest LOAEL in an intermediate-duration study was 0.006 mg/kg/day (only dose level tested) for maternal and developmental effects in mice (significant liver and kidney histopathology in dams and pups at weaning) dosed via the diet during gestation and lactation (Mansour et al. 2014). While no other study has examined the liver or kidneys from pups from mice exposed to endosulfan during gestation and/or lactation, intermediate- and chronic-duration studies (Hack et al. 1995; Hoechst 1984b; NCI 1978) have not reported histological alterations in the liver and kidneys from mice administered endosulfan via the diet in doses up to 3 orders of magnitude higher than those in the Mansour et al. (2014) study. Because of the inconsistency with results from other studies in mice, the Mansour et al. (2014) study will not be considered for MRL derivation; however, trying to replicate the results would be desirable. Aside from the Mansour et al. (2014) study, the lowest LOAEL for an intermediate-duration oral study was 0.61 mg/kg/day for a significant reduction in body weight in male rat pups (females were not used) on PND 21 born to Sprague-Dawley dams exposed to endosulfan by gavage during the entire period of gestation and lactation (Cabaleiro et al. 2008; Caride et al. 2010). A maternal dose of 6.12 mg/kg/day induced a reduction of approximately 45% in pup body weight on PND 21; no NOAEL was identified in these studies. The effect could have been due to poor milk production by the dams or to a direct action of endosulfan/metabolites on the pups via the milk, or both. It is worth noting that treatment of Wistar rats via the diet with a much higher dose of endosulfan, 29.8 mg/kg/day, on GDs 6–21 and during the lactation period resulted in a reduction of 11 and 4% in male and female pup weight, respectively, on PND 21, suggesting possible differences in strain sensitivity, but most likely the different mode of administration of the test material, gavage versus diet. In another study in rats, maternal doses of ≥0.61 mg/kg/day technical endosulfan by gavage during gestation and lactation resulted in alterations (increases and decreases) in the concentration and/or metabolism of dopamine, norepinephrine, and serotonin in the striatum from 60-day-old offspring (Lafuente and Pereiro 2013). Because no function was assessed, it is uncertain whether the 0.61 mg/kg/day dose level should be considered a NOAEL or LOAEL in this study. Altered sperm parameters and morphology of the testes were reported in mice dosed by gavage with 0.8 mg/kg/day technical endosulfan (the only dose level tested) for 3 weeks (Wang et al. 2012, 2014). A relative low LOAEL of 0.9 mg/kg/day was identified for decreases in humoral and cell-mediated responses in male Wistar rats (females were not tested)

administered endosulfan in the diet for up to 22 weeks; the NOAEL was 0.45 mg/kg/day (Banerjee and Hussain 1986). In this study, the humoral immune response was evaluated by measuring serum immunoglobulin concentration and antibody titer against tetanus toxoid; the cell mediated cell response was studied by leucocyte migration inhibition and macrophage migration inhibition tests. Slightly higher LOAELs of 1 mg/kg/day were established in a number of studies for a variety of end points. Singh et al. (2007b) reported increased incidence of myocardial fiber degeneration and degenerative changes in the liver and kidney from female Wistar rats dosed with 1 mg/kg/day technical endosulfan on GDs 6–20. The same group of investigators reported significant increases in the incidences of gross, visceral, and skeletal anomalies, and hepatocyte and renal tubule epithelial degeneration in GD 20 fetuses from the female Wistar rats mentioned above (Singh et al. 2007a, 2008). The same dose level induced degenerative changes in the endocrine pancreas and brain hemorrhage and edema in New Zealand white rabbits following 6 weeks of dosing (Mor and Ozmen 2010; Ozmen et al. 2010). In all of these studies, endosulfan was administered by gavage and only one dose level was tested; therefore, dose-response relationships could not be constructed. The remainder of the oral intermediate-duration studies tested higher doses of endosulfan. It is important to note that a study in Wistar rats, the same strain of rat used in the single dose level studies mentioned above, reported no significant compound-related alterations in hematology and clinical chemistry tests, body weight, ophthalmology, or gross or microscopic appearance of the liver, kidney, skeletal muscle, or nervous system tissues following administration of doses up to 37.2 and 45.5 mg/kg/day technical endosulfan via the diet to males and females, respectively, for 13 weeks (Sheets et al. 2004). This again indicates that the mode of administration of endosulfan plays a significant role in the manifestation of toxic effects of endosulfan. Of the studies mentioned above, the study by Banerjee and Hussain (1986) is selected for derivation of an intermediate-duration oral MRL for endosulfan. The selection is made on the basis that the study provided enough information to define a NOAEL and LOAEL and used the diet as the vehicle for administering endosulfan. Dosing via the diet is a more relevant route for environmental exposure of humans to endosulfan than gavage. With the exception of a study by Hoechst (1988c), which reported that doses up to 4.5 mg/kg/day given to Wistar rats 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, the study by Banerjee and Hussain (1986) is the only one that has examined immunocompetence in response to an infective agent, and would be helpful to try to replicate it. Vos et al. (1982) reported that serum levels of IgM and IgG were not significantly altered in male Wistar rats dosed with 5 mg/kg/day endosulfan for 3 weeks, but resistance to infection was not tested. Data from Banerjee and Hussain (1986) were considered for benchmark modeling analysis. However, only the information regarding serum levels of IgM and IgG, which are presented in a

table in the study, could have been subjected to benchmark modeling. Data regarding serum antibody titer to tetanus toxoid as well as leucocyte and macrophage migration inhibition were presented in figures from which only approximate values could be determined. Still, Banerjee and Hussain (1986) indicated in the figures the dose levels at which the responses were significantly different from controls. Therefore, since the lowest dose of 0.45 mg/kg/day (5 ppm in the food) was the NOAEL for serum IgG and IgM levels, antibody titer, and leucocyte and macrophage migration inhibition, the NOAEL/LOAEL approach is preferred for MRL derivation since it includes the three data sets. The intermediate-duration oral MRL for endosulfan is derived by dividing the NOAEL of 0.45 mg/kg/day by an uncertainty factor of 100 (10 for extrapolating from animals to humans and 10 for human variability). This yields an MRL of 0.005 mg/kg/day. A detailed description of the Banerjee and Hussain (1986) study is presented in Appendix A.

The intermediate-duration oral MRL of 0.005 mg/kg/day for endosulfan has also been adopted for the chronic-duration oral MRL based on the information summarized below. Chronic-duration dietary studies have been conducted in rats, mice, and dogs. Studies in Wistar rats were conducted by FMC (1959b) and Hoechst (1989a), the former used 25 rats per sex per group and the latter used 70 rats per sex per group. The results of Hoechst (1989a) were later published as Hack et al. (1995) with emphasis on the neoplastic effects of endosulfan. A 2-year study in NMRI mice was conducted by Hoechst (1988b) and the results were later published as Hack et al. (1995) also with emphasis on the neoplastic effects of endosulfan. A 2-year study in beagle dogs was conducted by FMC (1967) and a 1-year study was conducted by Hoechst (1989c); the former used four dogs per sex per group and the latter used six dogs per sex per group. NCI (1978) conducted long-term studies in Osborne-Mendel rats and B6C3F_1 mice. These studies conducted gross and microscopic examination of organs and tissues in addition to hematology and clinical chemistry tests. All of these studies used comparable doses of technical endosulfan (up to approximately 5 mg/kg/day) except for the NCI (1978) study that used doses considerably higher in rats (up to 48 and 22 mg/kg/day, in males and females, respectively). The lowest LOAELs in rats were identified in the Hoechst (1989a) study. The most salient findings in that study included reductions in weight gain and increased incidences of marked progressive glomerulonephrosis in male and female rats from the highest-dose groups. These data are presented in Tables 2-1 through 2-4. The incidence of aneurysms in the kidneys of male rats was also increased, but there was no dose-response relationship (10/70, 6/70, 17/70, 10/70, and 19/70 in the control and respective increasing dose groups).

Table 2-1. Incidence of Marked Progressive Glomerulonephrosis in Male Rats Exposed to Endosulfan for 2 Years

Dose (mg/kg/day)	Total number of rats	Number of rats with lesions
0	70	20
0.1	70	18
0.3	70	22
0.6	70	24
2.9	70	30[a]

[a]p=0.055

Source: Hoechst 1989a

Table 2-2. Incidence of Marked Progressive Glomerulonephrosis in Female Rats Exposed to Endosulfan for 2 Years

Dose (mg/kg/day)	Total number of rats	Number of rats with lesions
0	70	1
0.1	70	6
0.4	70	6
0.7	70	5
3.8	70	8[a]

[a]p=0.017

Source: Hoechst 1989a

Table 2-3. Data for the Change in Body Weight Gain in Male Rats Exposed to Endosulfan for 2 Years

Dose (mg/kg/day)	Number of animals tested	Weight gain (g)	Standard deviation
0	70	580	124
0.1	70	570	125
0.3	70	531	131
0.6	70	525	115
2.9	70	479[a]	94

[a]p<0.01

Source: Hoechst 1989a

Table 2-4. Data for the Change in Body Weight Gain in Female Rats Exposed to Endosulfan for 2 Years

Dose (mg/kg/day)	Number of animals tested	Weight gain (g)	Standard deviation
0	70	398	105
0.1	70	350	107
0.4	70	414	85
0.7	70	363	92
3.8	70	328[a]	100

[a]$p<0.05$

Source: Hoechst 1989a

In mice, the highest dose tested in the Hoechst (1988b) study, 2.9 mg/kg/day, caused a significant reduction in survival rate in females (28 versus 45% in controls). No other significant treatment-related effects were reported in chronic-duration studies in mice. No significant adverse effects were reported in the 2-year study in beagle dogs that received doses of endosulfan of up to 1 mg/kg/day via the diet (FMC 1967). In the 1-year study, the dogs were fed a diet containing 0, 3, 10, or 30 ppm endosulfan (0, 0.2, 0.7, or 2 mg/kg/day for males and 0, 0.2, 0.6, or 1.8 mg/kg/day for females) (Hoechst 1989c). Dogs fed a diet with ≥45 ppm endosulfan were sacrificed earlier due to severe neurological effects. In the 30 ppm group, three males and two females experienced violent contractions of the abdominal muscles and upper abdomen and convulsive movements of the chap muscles 2.5–6 hours after feeding. Dogs fed the ≤30 ppm diets did not show significant treatment-related alterations in organs and tissues or in hematology values. Among clinical chemistry parameters, dogs in the ≥10 ppm diets groups showed a significant increase in mean serum alkaline phosphatase activity relative to controls (up to approximately 2-fold) beginning at 1.5 months. In the absence of significant changes in other serum enzymes and lack of treatment-related histological alterations in the liver, the investigators did not consider the changes in alkaline phosphatase activity toxicologically significant.

Of the studies mentioned above, the 2-year study in rats conducted by Hoechst (1989a) is the most appropriate for MRL derivation based on the number of animals used per group (n=70), duration of exposure that covered the entire lifespan of the animals, and identification of valid end points, such as kidney lesions and body weight changes, for which dose-response relationships could be constructed. Data sets for marked progressive glomerulonephrosis and body weight changes in male and female rats reported in the Hoechst (1989a) study were analyzed using the BMD approach for MRL derivation. Models in the EPA BMDS (version 2.1.1) were fit to the four data sets to determine potential points of departure for the MRL. The data set for changes in weight gain in female rats proved not suitable for benchmark modeling even after dropping the two highest doses (out of five dose levels tested). Using the criteria for model selection mentioned earlier (see acute-duration oral MRL), the Log-logistic model (BMD_{10} 5.84 mg/kg/day; $BMDL_{10}$ 2.31 mg/kg/day) was selected as the best model to fit the incidence of marked progressive glomerulonephrosis in female rats. The Log-logistic model also provided the best fit for incidence of marked progressive glomerulonephrosis in male rats (BMD_{10} 1.17 mg/kg/day; $BMDL_{10}$ 0.56 mg/kg/day). The Exponential (Model 2) provided the best fit for the decrease in body weight gain in male rats (BMD_{10} 4.60 mg/kg/day; $BMDL_{10}$ 3.41 mg/kg/day). The lower $BMDL_{10}$ of 0.56 mg/kg/day is more health protective and is selected as the point of departure for MRL derivation. Applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the $BMDL_{10}$ of 0.56 mg/kg/day results in a chronic-duration oral MRL of 0.006 mg/kg/day for endosulfan.

Since this MRL is slightly higher than the intermediate-duration oral MRL of 0.005 mg/kg/day derived for endosulfan, the intermediate-duration oral MRL, which is protective of potential effects due to chronic exposure to endosulfan, is adopted also as the chronic-duration oral MRL for endosulfan.