Regulatory Toxicology and Pharmacology 56 (2010) 4–17

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# Human health risk assessment of endosulfan. I: Toxicology and hazard identification

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ARTICLE INFO

*Article history:*
Received 8 July 2009
Available online 3 September 2009

*Keywords:*
Endosulfan
Risk assessment
Critical NOEL/NOAEL
LOEL/LOAEL
FQPA
Hazard identification

ABSTRACT

Endosulfan is persistent in the environment and toxic to wildlife. Legal mandates necessitate that a risk assessments be performed for endosulfan by the California Department of Pesticide Regulation (CDPR) and the United States Environmental Protection Agency (USEPA). This hazard identification (hazard ID) compared critical no-observed effect levels (NOEL) for acute, subchronic and chronic exposure intervals between the agencies. NOELs were discussed in light of their application to numerous exposure scenarios (occupational, general population and dietary). Only the acute oral NOELs differed between CDPR (0.7 mg/kg/day) and USEPA (1.5 mg/kg/day). Pregnant rabbits were considered by CDPR to be more responsive to low gavage doses of endosulfan than non-pregnant female or male rats in the acute study selected by USEPA. NOELs for other exposure routes and durations were similar between agencies. CDPR and USEPA concurred that a Food Quality Protection Act (FQPA, 1996) Safety Factor is not needed after evaluating all studies including a Developmental Neurotoxicity study. The SF was reduced to 1×. NOELs generated from this hazard ID will be used to calculate the Margins of Exposure for all scenarios and subsequently the risk characterization for endosulfan.

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## 1. Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), patented in 1956 (Ware, 1994), is an organochlorine pesticide consisting of two isomers (α: 64–67%; β: 29–32%; Maier-Bode, 1968; NRCC, 1975). The α-isomer is more toxic to insects and mammals than the β-isomer.

Endosulfan binds to and blocks the $Cl^-$ channel linked to the γ-amino-butyric acid ($GABA_A$)-receptor. It does not affect the GABA recognition site and so can be termed a "non-competitive GABA antagonist" (Lawrence and Casida, 1984; Abalis et al., 1986; Ffrench-Constant, 1993). Because $GABA_A$ receptors are the principal inhibitory neuroreceptors in the mammalian brain, the antagonism of GABAergic neurons within the central nervous system causes generalized brain stimulation (Abalis et al., 1986; Cole and Casida, 1986; Gant et al., 1987; Ozoe and Matsumura, 1986). When GABA binds to its receptor, the $Cl^-$ ion channels are opened, leading to an influx of $Cl^-$ into neurons through an electrochemical gradient. The result is hyperpolarization of the cell membrane and inhibited neuron firing. Endosulfan prevents $Cl^-$ from entering neurons, thereby blocking the postsynaptic effect of GABA binding to its $GABA_A$ receptor, resulting in uncontrolled excitation.

As one of the few organochlorines still registered for use, endosulfan has elicited worldwide concern for many reasons. Although endosulfan serves as a contact and stomach insecticide for food and non-food crops, it is also toxic to fish and other aquatic organisms (Suntio et al., 1988; Naqvi and Vaishnavi, 1993; Toledo and Jonsson, 1992). Endosulfan is considered a Persistent Organic Pollutant (IPEN, 2008) throughout the world because it has been detected in European lakes (Carrera et al., 2002) as well as in the Arctic (eastern Russia and western Canada) in air, rain, snow, ice and fine particulate, water ways of lakes, river, sea, sediment, soil, plants, and generally in acid media (Fan, 2008; USEPA, 2002; Usha and Harikrishnan, 2005). Non-governmental organizations such as Pesticide Action Network of North America (PANNA, 2008) have campaigned for a total worldwide ban of endosulfan due to concerns that it is a carcinogen, teratogen and a male reproductive toxicant. In California, and throughout the U.S., endosulfan is still widely used on many crops and exposure data show that the general population is exposed via diet and air (USEPA, 2007b; Beauvais et al., 2009a,b; Silva and Carr, 2009). There is also ample evidence that endosulfan is acutely poisonous to humans through accidental and intentional exposure as documented in California by Beauvais et al. (2009a,b), in the United States (USEPA, 2002), Canada (Health Canada, 2007) and throughout the world (WHO, 1998) and the effect observed generally is neurotoxicity.

No pesticide can be used in the U.S. unless it is registered by the United States Environmental Protection Agency (USEPA) and registration involves performing a comprehensive human health risk assessment. However, individual states such as California may

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0273-2300/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.yrtph.2009.08.013

perform a parallel risk assessment for a given pesticide registration based on legislation or other conditions specific or unique to that state. California, for example is the number 1 agricultural state in the U.S. with 1/3rd of its 100 million acres devoted to agriculture. A parallel risk assessment for endosulfan was considered necessary by the California Department of Pesticide Regulation (CDPR) based on (1) low No-Observed-Effect-Levels (NOEL) in a rat inhalation, a rabbit teratology and a chronic dog study signaling a potential for unacceptable risks for human exposures, based on current product labels; (2) current endosulfan use under use regimes allowed in California since several endosulfan product labels have California-specific application directions; and (3) potential effects due to applications in California's unique climatic and cultural conditions. With regard to climate, pesticide residues which dissipate readily under warm, humid conditions typical of growing seasons elsewhere may persist longer under hot, dry conditions that occur in much of California during the growing season. For many crops, growing seasons are longer in California than in other parts of the U.S. and (4) California has laws intended to limit ambient air concentrations of pesticides, including the Toxic Air Contaminants Act (1983; California Health and Safety Code, Sections 39650-39761), which codifies the state program to evaluate and control toxic air contaminants (TACs), including pesticides. A risk assessment was deemed necessary as endosulfan, based on preliminary exposure estimates, had the potential to be listed as a TAC.

In a series of four articles, the risk assessment for endosulfan is described, including Part I: The Toxicology and Hazard Identification (hazard ID), Part II. Dietary Exposure (Silva and Carr, 2009); Part III: Occupational Handler Exposure and Risk (Beauvais et al., 2009a); and Part IV. Occupational Reentry and Public Non-Dietary Exposure and Risk (Beauvais et al., 2009b). This article will focus on Part I. The selection of endpoints and definitive studies for each exposure interval will be compared and discussed in light of their application to the numerous exposure scenarios in Parts II–IV (occupational, general population and dietary) for CDPR and USEPA. The exposure estimates, appraisal of risk and uncertainties involved in the risk characterization for endosulfan will be provided in accompanying articles (Beauvais et al., 2009a,b; Silva and Carr, 2009).

## 2. Materials and methods

### 2.1. Regulatory mandates for pesticide risk assessment

Endosulfan is registered in California and by USEPA; however certain federal mandates as well as specific requirements in California must be fulfilled before a pesticide can be used in California. Many data requirements overlap between the CDPR and USEPA.

#### 2.1.1. Pesticide registration CDPR and USEPA

CDPR performs risk assessments for currently registered pesticides under the California Code of Regulations Title 3, Section 6158 (3 CCR 6158) such that exposures by all routes associated with all uses are evaluated to protect human health (occupational and general population). The toxicology data requirements related to endosulfan's continued registration for the respective agencies (e.g. California Toxic Air Contaminants Act, 1989; California Birth Defects Prevention Act, 1994; California Food Safety Act, 1989; Federal Insecticide, Fungicide and Rodenticide Act, (USEPA, 1997), Food Quality Protection Act (FQPA), 1996) were satisfied. The Toxic Air Contaminants Act (1983), mentioned above, lists a pesticide as a TAC if the concentrations in ambient air are within an order of magnitude of the concentration that has been determined to be adequately protective of human health.

NOELs from oral gavage or diet studies were used, when needed, as surrogates for dermal studies by CDPR and USEPA, since no acceptable dermal studies were available. This was possible because oral and dermal toxicity are similar and a dermal penetration factor is available (Craine, 1988). Studies submitted by registrants to fill data requirements were performed according to Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Guidelines (USEPA, 1998, 2007a).

#### 2.1.2. USEPA pesticide re-registration

In contrast to CDPR, USEPA performs risk assessments to meet requirements of FIFRA; for example, FIFRA Section 2(bb), codified in the U.S. Code at Title 7, Section 136,(2)(bb), requires USEPA to determine that allowed pesticide uses do not cause "unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits." USEPA has a program to review older pesticides, such as endosulfan under FIFRA (USEPA, 1997) to ensure that they meet current scientific and regulatory standards. The USEPA was required to perform a risk assessment for endosulfan as part of the pesticide re-registration process. Unlike California's Toxic Air Contaminants Act, FIFRA does not specifically require USEPA to estimate ambient air exposures to pesticides.

### 2.2. Endosulfan risk assessments (CDPR and USEPA)

A risk assessment for a pesticide is prepared in the form of a risk characterization document (RCD) by CDPR, and the occupational and residential risk assessment prepared by USEPA supports a Re-registration Eligibility Decision (RED). Each document includes data on toxicology, environmental fate, exposure and human experience with the pesticide. The approach used by both agencies is consistent with that described by the National Research Council (NRC, 1983). The process involves four parts: (1) hazard identification (hazard ID), (2) dose–response assessment, (3) exposure assessment and (4) risk characterization. The risk characterization integrates the first three parts into an evaluation of the risk. A large portion of the characterization consists of an appraisal of the significance of the findings along with the uncertainties involved in the decision process.

Under the FQPA (1996), the USEPA was mandated to evaluate endosulfan where an extra 10-fold (10×) safety factor (SF) may be used to account for potential reproductive or developmental toxicity (unless it is decided, based on reliable data, that a different margin would be safe). Available information on: (1) aggregate exposure from all non-occupational sources; (2) effects of cumulative exposure to the pesticide and other substances with common mechanisms of toxicity; (3) effects of *in utero* exposure; and (4) potential for endocrine disruption were considered by USEPA (USEPA, 2002). This article will discuss an evaluation of the effects of *in utero* exposure and the potential for endocrine disruption in animal studies.

As of 2002, the RED for endosulfan indicated that the 10× SF would be retained since open literature studies showed male reproductive and developmental toxicity in rats (USEPA, 2002). Some of the parameters measured in these studies had not previously been required under FIFRA Guidelines prior to their 1998 FQPA revision (USEPA, 1998). Endpoints such as sperm morphology, motility and production, vaginal opening, preputial separation (PPS), and others were added (USEPA, 1998). Registrants were required to perform a dietary Developmental Neurotoxicity study (DNT, Gilmore et al., 2006) to evaluate both the potential for neuropathology and/or neurobehavioral toxicity to pups after endosulfan exposure *in utero* or through milk. The DNT study examined the reproductive parameters that had not been included in the earlier, registrant-submitted rat reproduction study (Edwards et al., 1984).

Since endosulfan is primarily a neurotoxin both acute and subchronic neurotoxicity studies were also required by the USEPA (2002).

*2.2.1. Hazard ID (USEPA, 2009)*

In order to identify the potential adverse effects of endosulfan (hazard ID) a combination of toxicity studies in the open literature and reports submitted by the principal registrants are evaluated to obtain critical endpoints. Both CDPR and USEPA reviewed registrant-submitted and open literature studies to obtain critical toxicity endpoints. More weight is generally placed on FIFRA Guideline studies by both agencies. The lowest dose that does not cause toxicity (NOEL or no-observed-adverse-effect-level: NOAEL as designated by USEPA) is identified as the critical NOEL to address acute, subchronic or chronic exposure durations. Ultimately the NOEL is used to estimate safe levels of exposure occupationally, to the general population or in diet.

*2.2.2. Exposure intervals for toxicity tests vs. human exposure estimates*

The treatment intervals for toxicity tests are, in general, depending on species tested and route administered: acute (single dose), subchronic (21–90 d depending on route) or chronic (1–2 years) and they are the intervals from which the NOELs for hazard ID are selected. These intervals are different in terminology and duration, when applied to human exposure (non-dietary: occupational or to the general public) and they also differ somewhat between CDPR and USEPA. CDPR defines human exposure as short-term (<1 week), seasonal (>1 week but < 1 year; constant or intermittent) and annual (exposure throughout the year) for dermal/oral and inhalation scenarios. USEPA defines human occupational intervals as short-term (1–30 d), and intermediate-term (1–6 months) for dermal and inhalation scenarios and long-term (>6 months) for dermal scenarios (USEPA, 2007b). The dietary exposure intervals for each agency (generally "acute" and "chronic") are described in detail in an accompanying article (Silva and Carr, 2009).

*2.2.3. Exposure estimates*

Estimates of theoretical exposure are calculated for dietary, dermal (occupational or child/adult swimmers in surface water), inhalation (occupational or air for infant/adult bystanders at application sites) and aggregate (dietary + [occupational: dermal/air] or [public: swimmer/air]) values. The methods and data analyses for CDPR and USEPA are described and compared in accompanying publications (Beauvais et al., 2009a,b; Silva and Carr, 2009).

*2.2.4. Risk characterization*

The NOELs in this article were used for margin of exposure (MOE: ratio of NOEL to exposure levels; to quantify potential risk) estimates by CDPR for occupational (dermal), dietary, swimmers in surface water (child/adult: dermal and non-dietary ingested) and inhalation by the general population (infant/adult bystanders at application sites). For USEPA, the NOAELs were used to characterize risk in occupational (dermal), inhalation and dietary scenarios (USEPA, 2004, 2009).

## 3. Results

*3.1. Pharmacokinetics*

The majority of absorbed endosulfan, regardless of exposure route in the rat, is excreted rapidly in feces, with virtually no retention in tissues, despite the lipophilicity of endosulfan and its primary metabolite, endosulfan sulfate (Dorough et al., 1978). Elimination via the enterohepatic circulation was not a major route for endosulfan metabolism. At 120 h, 88% of α-[$^{14}$C]-endosulfan and 87% of β-[$^{14}$C]-endosulfan had been eliminated. Chan et al. (2005) showed $^{14}$C-endosulfan-derived radioactivity in blood had a distribution half-life of 31 min and a terminal elimination half-life of 193 h. Blood concentration reached its maximum (0.36 mg/L) at 2 h post-dosing after being rapidly absorbed from the GI tract (absorption rate constant = 3.07 $h^{-1}$). After endosulfan was dermally administered to rats, within 5 days a dermal penetration factor of 47.3%, as estimated by CDPR (USEPA used 45%), was achieved and 95% of the absorbed material was eliminated (Craine, 1988). For oral and inhalation exposure scenarios, both CDPR and USEPA assumed 100% absorption.

After oral treatment in rats with of [$^{14}$C]-endosulfan, liver and kidney were the sites of greatest radioactivity. These are likely the primary sites of biotransformation since the concentrations and activities of xenobiotic metabolizing enzymes such as P450s and glutathione-transferases (GST) were also increased (Bebe and Panemangalore, 2003; Fabacher et al., 1980; Siddiqui et al., 1987; Singh and Pandey, 1989a,b, 1991; Tyagi et al., 1984). Stereoselective endosulfan sulfate formation from human recombinant P450s showed that α-endosulfan metabolism is mediated by CYP2B6, CYP3A4 and CYP3A5 and the β-isomer is mediated by CYP3A4 and CYP3A5 (Lee et al., 2006). Fig. 1 shows a proposed metabolic pathway for endosulfan.

*3.2. Hazard identification*

The definitive studies, described below for the critical acute, subchronic and chronic NOELs were used in the calculation of MOEs (risk characterization) for all exposure scenarios. Database summaries are available for CDPR (Silva, 2008) and USEPA (USEPA, 2000, 2002, 2007b).

*3.2.1. Acute toxicity*

*3.2.1.1. CDPR definitive study for the oral/dermal NOEL (rabbit developmental toxicity: Nye, 1981) Table 1.* For both the oral and dermal endpoints, the lowest NOEL identified by CDPR was in a rabbit developmental toxicity study (Nye, 1981). Mated female New Zealand White rabbits were gavaged with endosulfan at 0, 0.3, 0.7 or 1.8 mg/kg/day during gestation days (GD) 6–28. The maternal NOEL was 0.7 mg/kg/day based on increased mortality and on clinical signs which occurred during treatment. Clinical signs began on day 6 (treatment day 1) at 1.8 mg/kg/day, signifying an acute effect. There was no developmental toxicity at any dose.

There were no acceptable acute dermal studies available to establish a dermal endpoint, therefore the definitive acute oral NOEL (0.7 mg/kg/day; Nye, 1981) was used as a surrogate for MOE calculations.

*3.2.1.2. USEPA definitive study for the acute oral NOAEL (neurobehavioral toxicity; Bury, 1997) Table 1.* The USEPA used an acute oral rat neurobehavioral toxicity study for the dietary risk characterization that applied to the general population. Endosulfan was administered by oral gavage in a single dose to fasted Wistar rats at 0, 6.25, 12.5, 25, 50 or 100 mg/kg/day (males, M) and 0, 0.75, 1.5, 3, 6 or 12 mg/kg/day (females, F). The neurotoxicological screening (Functional Observational Battery, FOB and motor activity, MA) was performed 7 days prior to treatment initiation, 8 h post-dosing (time of peak effect) and at 7 days and 14 days post-dosing. Three weeks post-dosing animals were terminated for neuropathological examination. The NOAEL was 12.5 mg/kg/day (M) and 1.5 mg/kg/day (F), based on an increase in clinical signs in males at 25 mg/kg/day and greater and in females at 3 mg/kg/day and greater, lasting for 1 day. Like the oral and dermal $LD_{50}$ studies and the inhalation $LC_{50}$ study, this study showed females as the more sensitive

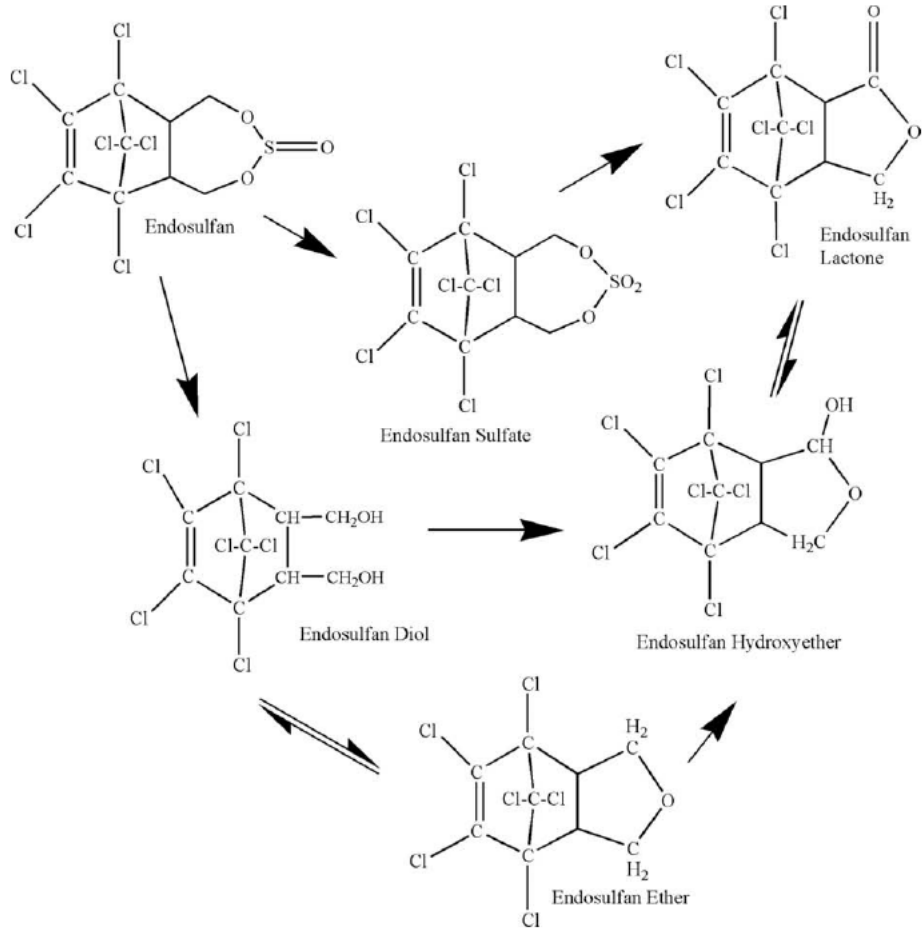


**Fig. 1.** Proposed metabolic pathway in rat and sheep for endosulfan (Dorough et al., 1978; Gorbach et al., 1968; Bebe and Panemangalore, 2003; Lee et al., 2006). *Notes:* Phase I reactions on endosulfan are performed with P450s: CYP2B6, CYP3A4 and CYP3A5; Phase II reaction is with GST. Other enzymes involved with endosulfan metabolism are antioxidants: SOD, GPX and catalase.

**Table 1**
Acute definitive studies for the critical endpoints selected by CDPR and USEPA.

| Species | Exposure | Effect | NOEL/NOAEL | LOEL/LOAEL | References |
|---|---|---|---|---|---|
| *Oral* | | | | | |
| Rabbit | pregnant[a] | 12 days gavage | Maternal death, clinical signs at treatment day 1 at the highest dose tested (HDT) | Dam: 0.7 mg/kg/day | 1.8 mg/kg/day HDT |
| Nye (1981) | | | | | |
| Rat M/F[b] | Single gavage | Death, clinical signs, neurotoxicity | M 12.5 mg/kg/day<br>F 1.5 mg/kg/day | M 25 mg/kg/day<br>F 3.0 mg/kg/day | Bury (1997) |
| *Inhalation $LC_{50}$* | | | | | |
| Rat M/F[a,b] | 6 h/d, 5 d/weeks, 21 d, Nose Only | Clinical signs, ↓ body weight gain; ↓ food/water consumption; clinical chemistry parameters affected | 0.194 mg/kg/day (0.001 mg/L) | 0.387 mg/kg/day (0.002 mg/L) | Hollander et al. (1984) |

[a] Selected by CDPR as the definitive study.
[b] Selected by USEPA as the definitive study.

gender (Table 1). The USEPA used the lower, female NOEL (1.5 mg/kg/day) for the acute MOE estimate.

*3.2.1.3. USEPA acute "dermal" (developmental neurotoxicity, DNT; Gilmore et al., 2006).* For the short-term dermal occupational exposure (1–30 d) an acceptable dermal toxicity study was not available. The USEPA selected the rat DNT study as a surrogate for dermal MOE estimates to apply to a high risk occupational group (female workers). In that study, endosulfan was fed in the diet to mated female Wistar rats at 0, 3.74, 10.8, and 29.8 mg/kg/day from GD 6 through lactation day (LD) 21 (Gilmore et al., 2006). Offspring from 23 litters at 0, 3.73 and 10.8 mg/kg/day and pups from 21 litters at 29.8 mg/kg/day were assessed neurologically up to postnatal day (PND) 75 in the FOB, in MA, auditory startle response, passive avoidance learning and memory and water maze learning and memory assessments. The motility, numbers and morphology of sperm from male pups were evaluated. Neuropathologic examinations and morphometric analyses of selected neurological tissues from the pups were performed. The mean body weight of the dams was decreased in a dose-related manner during gestation

**Table 2**
Subchronic Definitive Studies for the Critical Endpoints Selected by CDPR and USEPA.

| Species | Exposure | Effects | NOEL/NOAEL | LOEL/LOAEL | Reference |
|---|---|---|---|---|---|
| *Oral* | | | | | |
| Rat M/F | 2-Generation diet (CDPR) | Maternal: ↑ liver and kidney weight<br>Pup: ↓ body weights | 1.18 mg/kg/day | 5.4[b] mg/kg/day | Edwards et al. (1984) |
| RatM/F | Developmental Neurotoxicity Diet (USEPA) | Maternal: ↓ body weight, food intake, food efficiency<br>Pup: ↓ body weight and weight gain, delayed PPS | 1.25 mg/kg/day (estimated NOAEL)[c] | 3.74 mg/kg/day | Gilmore et al. (2006) |
| *Inhalation (21 treatments, 6 h/d, 5 d/weeks over 29 d)* | | | | | |
| Rat M/F | Nose only (CDPR and USEPA) | Clinical signs, ↓ body weight gain; ↓ food/water consumption; clinical chemistry parameters affected | 0.194[a] mg/kg/day (0.001 mg/L) | 0.387 mg/kg/day (0.002 mg/L) | Hollander et al. (1984) |

[a] CDPR used 0.194 mg/kg/day and USEPA rounded up to 0.2 mg/kg/day for the inhalation NOEL values.
[b] The NOELs are expressed as mean dosages of F0 + F1b generations, each, for males and for females. The lowest of the NOELs was used as the critical NOEL.
[c] Estimated by 1/3rd LOAEL.

and this decrease persisted through lactation with the mean body weight of the dams at 10.8 mg/kg/day and greater significantly lower than controls through LD 7. The mean food consumption was likewise affected for all of the treatment groups during gestation. The report stated that the decrease in food consumption, while transitional, was likely due to palatability. The mean body weights of the pups in all of the treatment groups during lactation were decreased but there was no treatment-related effect on fetal gestation time, live births, viability, or lactation indices. Preputial separation (PPS) was marginally delayed (4–5%) in males at 10.8 mg/kg/day and greater (0 = 44.9 d; 3.74 mg/kg/day = 44.8 d; 10.8 mg/kg/day = 47.1 d; $p < 0.05$ at 29.8 mg/kg/day = 46.8 d). There were no effects on other developmental parameters, neurobehavioral parameters, and neuropathology or morphometric analyses of brain in animals evaluated. The maternal "lowest observed adverse effect level" (LOAEL) was 3.74 mg/kg/day, based upon lower mean body weights (5–6%) and lower food consumption (12%). While these decreases are marginal, they are dose-related and therefore considered potential adverse effects. The developmental LOAEL was also 3.74 mg/kg/day based upon the lower mean pup body weights (↓8%) occurring only on PND 11 at the lowest dose tested (LDT).

Since a NOAEL was not obtained, the USEPA extrapolated from the LOAEL to NOAEL (LOAEL = 3.74 mg/kg/day) to obtain an estimated "NOAEL" value as a surrogate for dermal toxicity (USEPA, 2007b). The factor used for this determination was 3×, ("NOAEL" = 3.74 mg/kg/day ÷ 3 = 1.25 mg/kg/day) based on dam and pup weight loss.

*3.2.1.4. CDPR and USEPA acute inhalation (short, 1–30 d exposure).* An acute inhalation ($LC_{50}$) study was performed in rat; however, a NOEL/NOAEL was not achieved (LDT = 0.61 mg/kg; 0.0036 mg/L; Hollander and Weigand, 1983). It was acceptable however, for CDPR to use a subchronic NOEL in lieu of an acute NOEL as has been described by USEPA (2004). The doses selected for the definitive subchronic inhalation study were based the results of the acute inhalation $LC_{50}$ study as well as on the results of a rangefinding study (Hollander and Weigand, 1983; Hollander et al., 1984). In the subchronic rangefinding study, Wistar rats were treated 7 times at 0.0024 mg/L (0.44 mg/kg/day, LDT) and 0.0065 mg/L (1.11 mg/kg/day) to determine the doses for the definitive study (Hollander et al., 1984). Results showed that clinical signs of neurotoxicity occurred both during and after dosing. Death and decreased body weights were observed at both doses. Since effects were observed at 0.0024 mg/L (0.44 mg/kg/day, LDT), the investigators decided that 0.002 mg/L (0.387 mg/kg/day) would be the highest concentration for the subchronic definitive study. The definitive subchronic rat inhalation study (21 doses over 29 days), with a NOEL of 0.194 mg/kg/day (0.0010 mg/L; USEPA rounded to 0.2 mg/kg/day), was used by both CDPR and USEPA to calculate the MOEs for acute single-day and short-term occupational exposure (Hollander et al., 1984). Details of this study are described in Section 3.2.2.3 (CDPR and USEPA: subchronic inhalation NOEL/NOAEL), below.

*3.2.2. Subchronic toxicity*

*3.2.2.1. CDPR definitive study for the subchronic oral/dermal NOELs (2-generation reproduction; Edwards et al., 1984) Table 2.* The 2-generation rat reproduction study was used by CDPR for the subchronic oral MOE estimations and as a surrogate for dermal MOE estimates. Crl:COBS(CD)BR rats were fed endosulfan in diet and each parental generation was mated twice (Edwards et al., 1984). Treatment was equivalent to 0.02 1.0, 4.99 mg/kg/day (F0 M), 0.24, 1.23, 6.18 mg/kg/day (F0 F) and 0.23, 1.18, 5.72 mg/kg/day (F1b M) and 0.26, 1.32, 6.92 mg/kg/day (F1b F). Developmental endpoints were neonatal body weights, sex ratio, pup mortality, total litter loss, resorptions (early and late), gross morphology, litter size, anomalies, malformations and others. There were no clinical signs of neurotoxicity. F1a pups had a slight decrease in mean litter weight on PND 12 (7%) and PND 21 (9.7%) and F1b litter weight was decreased 12% at the highest dose tested (HDT). Adults (F0 F; F1 M/F) had a marginal decrease in body weight gain and food consumption (F1 M), and an increase in relative liver (F0 M, F0 and F1b F) and kidney weights (F0 and F1b M) at the HDT. Body weight decrease in pups at PND 12 to 21 is expected at the higher treatment levels because (1) pups are transitioning from nursing to eating solid food; (2) decreased palatability of treated diet and (3) increased doses for pups as they receive endosulfan in diet and through milk. A dose response was not observed for pituitary, ovarian or uterine weight effects and they were considered to be incidental. The lowest of the NOELs for each generation was the critical value. The critical systemic, reproductive and pup NOEL used by CDPR was 1.18 mg/kg/day.

*3.2.2.2. USEPA definitive study for subchronic dermal NOAEL (USEPA, 2002, 2007b) Table 2.* According to the USEPA Health Effects Division's Addendum and Update to the 2002 Risk Assessment Decision, an oral NOAEL for subchronic duration was not selected (USEPA, 2007b). For the "intermediate-term" dermal occupational exposure (1–6 months) an estimated NOAEL (1.25 mg/kg/day) based on the LOAEL = 3.74 mg/kg/day from the rat DNT study (described above in Section 3.2.1.3) was used to estimate the MOE.

*3.2.2.3. CDPR and USEPA: subchronic inhalation NOEL/NOAEL: Hollander et al., 1984 (Table 2).* Both agencies selected the subchronic rat inhalation study for the critical NOEL. In this study, endosulfan was administered by inhalation (aerosol, nose only) 21 times over 29 days (Monday–Friday, 6 h/d) to adolescent (4–6 weeks) Wistar

**Table 3**
Chronic definitive studies for the critical endpoints selected by CDPR and USEPA.

| Species | Exposure | Effect | NOEL/NOAEL[a] | LOEL/LOAEL[a] | References |
|---|---|---|---|---|---|
| *Oral* | | | | | |
| DogM/F | 1 year diet (CDPR) | Premature termination, clinical signs of neurotoxicity; ↓ body weight gain; ↓ food intake | 0.57 mg/kg/day | 1.98 mg/kg/day | Brunk (1989) |
| RatM/F | 104 week diet (USEPA) | Aneurysms, glomerulonephrosis and nephritis; enlarged kidneys, proteinuria; ↓ body weight gain | 0.6 mg/kg/day | 2.9 mg/kg/day | Ruckman et al. (1989) |
| *Inhalation (21 treatments, 6 h/d, 5 d/weeks over 29 d)* | | | | | |
| Rat M/F | Nose only (CDPR and USEPA) | Clinical signs, ↓ body weight gain; ↓ food/water consumption; clinical chemistry parameters affected | 0.194[a] mg/kg/day(0.001 mg/L) | 0.387 mg/kg/day(0.002 mg/L) | Hollander et al. (1984) |

[a] Represents lowest mean value for the corresponding dose based on approximations of endosulfan intake in diet.

rats at 0 (air only), 0 (ethanol–polyethylene 400 (1:1)), 0.0005, 0.0010 and 0.0020 mg/L air (0.097, 0.194 and 0.387 mg/kg/day; Hollander et al., 1984). Following the 29-day treatment period, some were terminated and some were observed over a 29-day recovery period. The NOEL/NOAEL was 0.0010 mg/L (0.194 mg/kg/day). At 0.0020 mg/L (0.387 mg/kg/day) males showed clinical signs, decreased food/water consumption and decreased body weight gain. Clinical chemistry in females showed significant effects in chloride, calcium, creatinine and SGOT at 0.0020 mg/L (0.387 mg/kg/day). There were no histopathological effects or gross morphological effects observed in either sex. All effects were reversed by recovery day 29 except for 1 male at HDT with neurotoxicity. CDPR applied the NOEL (0.194 mg/kg/day) to the seasonal inhalation MOE estimates for occupational scenarios and for infant and adult bystanders at application sites. The USEPA used a NOAEL of 0.2 mg/kg/day for the "intermediate-term" inhalation occupational exposure (1–6 months).

### 3.2.3. Chronic toxicity

*3.2.3.1. CDPR definitive study for the chronic oral/dermal NOEL (Brunk, 1989) Table 3.* CDPR selected the dietary NOEL from the dog study to determine both the oral and dermal chronic MOEs (no chronic dermal study was available) since this study provided the lowest NOEL of all acceptable chronic studies. Endosulfan was fed to Beagle dogs at 0, 0.22, 0.57, 2.09, and 2.2/3.08/3.7 mg/kg/day (M) and 0.19, 0.65, 1.98, and 1.95/2.78/3.57 mg/kg/day (F) for 1 year (Brunk, 1989). At the HDT, dogs were treated at increasingly higher doses throughout the study. One male at 2.09 mg/kg/day was killed *in extremis* on day 126, after 125 treatments. All dogs at HDT were sacrificed on days 146–147, due to an onset of clinical signs of neurotoxicity after the dose was increased to 3.7 mg/kg/day (M) and 3.57 mg/kg/day (F). One male at 2.09 mg/kg/day and one male at 3.7 mg/kg/day were terminated on days 276 and 126, respectively, due to poor condition. On the morning of the 136th day, after 135 applications, one female, at HDT showed severe clinical signs. Since these reactions occurred between 3 p.m. and 7 a.m., the dogs in all groups were subsequently treated at an earlier hour. Starting 2.5–6 h after treatment there were sudden and violent contractions of the abdominal muscles, and convulsive movement of the chaps, though not followed by vomiting. Neurological symptoms, having to do with reflexes, were noted only at termination. Decreased body weights were observed in males at 2.09 mg/kg/day and higher beginning week 44. Both sexes showed a temporary decrease in percent of food consumed at the mid and high doses. The lowest NOEL was 0.57 mg/kg/day in males was used by CDPR for chronic dietary, occupational and swimmer in surface water MOE estimates.

*3.2.3.2. CDPR chronic inhalation NOEL ("estimated NOEL").* A chronic inhalation study was not performed for endosulfan. Therefore the NOEL had to be estimated for inhalation scenarios involving chronic occupational exposure or long-term exposure to the general population near application sites. A NOEL was obtained by dividing the subchronic NOEL by a 10× uncertainty factor (UF) for extrapolation from a subchronic to a chronic interval to produce an "estimated NOEL" (estimated NOEL = [0.194 mg/kg/day - 10× UF] = 0.0194 mg/kg/day) (Hollander et al., 1984; USEPA, 2004).

*3.2.3.3. USEPA definitive study for the chronic oral NOAEL (combined: chronic/ oncogenicity, Ruckman et al., 1989) (Table 3).* The definitive chronic oral study selected by USEPA was used to estimate dietary MOEs for long-term (> 6 months) exposures. Crl:CD (SD) BR rats were fed endosulfan in the diet for 104 weeks at 0, 0.1, 0.3, 0.6 or 2.9 mg/kg/day (M) and 0.1, 0.4, 0.7 or 3.8 mg/kg/day (F) to evaluate oncogenicity and chronic effects (Ruckman et al., 1989). Also treated for 104 weeks was a satellite group intended for blood sampling at prescribed intervals (no interim sacrifices were done in this group). Bodyweight gain was decreased 8–18%, compared to controls in both sexes at HDT. Absolute testes weights appeared to decrease at the HDT but were within the historical control range. There were no differences in relative testes weights at any dose and despite the decreases in body weight gain, the body weights were within historical control range. Kidney enlargement occurred at 3.8 mg/kg/day (F). Progressive glomerulonephrosis was increased in both sexes at the HDT and was stated in the report to be a common, age-related, spontaneously occurring renal disease associated with proteinuria (especially in males). There was an increased incidence of aneurysms in blood vessels at 2.9 mg/kg/day, which primarily affected the pancreas, mesentery and/or liver after week 80 in males. There was a slight increase in the incidence of pituitary adenomas at 2.9 mg/kg/day (M) but no dose-related trend. USEPA considered this observation to be an incidental effect. The chronic NOAEL of 0.6 mg/kg/day was used for dietary risk characterization.

*3.2.3.4. USEPA chronic dermal and chronic inhalation intervals.* Studies for long-term (>6 months) dermal and inhalation exposures were not selected because for USEPA purposes "Long-term dermal exposure is not expected..." (USEPA, 2007b).

### 3.2.4. Cholinesterase effects

Cholinesterase (ChE) activity was examined in several reports: Ansari et al., 1987; Barnard et al., 1985; Castillo et al., 2002; Kushwah and Dikshith, 1981; Seth et al., 1986). Results indicate that endosulfan does not have a consistent effect on ChE. In the cases where effects are observed, doses used are highly toxic and any "effect" observed is likely incidental. Additionally, all NOELs used by CDPR and USEPA are substantially below any that have been shown to induce an effect on ChE and therefore, ChE is not

considered to be a toxicologically relevant endpoint for the purpose of this risk assessment.

*3.2.5. Human epidemiology*

*3.2.5.1. Children.* An epidemiological study was performed to assess potential effects of aerial spraying of endosulfan on sexual maturation rate (SMR) in children (Saiyed et al., 2003). Endosulfan was the only pesticide used on cashew nut plantations in Kerala, India where school children (aged 10–19 years) were exposed via air, water (runoff) and soil. A reference group of children was from a village 20 km away without any history of aerial endosulfan spraying. Endosulfan blood serum residues were increased (higher than background levels) in study males, compared to the reference group, and there was a decrease in SMR for pubic hair, testicular and penis development. The authors indicated that a follow-up study was to be performed with a larger sample size to further investigate those initial findings. Indulkar (2004) and Abraham (2004) countered Saiyed et al. (2003) by pointing out the study deficiencies (e.g. use of pesticides in "reference" areas; small study sample size; only 1 blood sample/subject; no description of normal biological range for SMR or hormone levels in that population; raised serum endosulfan levels inconsistent with lower background levels given endosulfan is rapidly cleared and does not bioaccumulate). Saiyed et al. (2003) proposed that a follow-up should be performed on the children to assess possible long-term effects and a repeat study should also be performed with a larger sample size to validate the study findings.

*3.2.5.2. Transmission of endosulfan from mother to child.* Women of reproductive age and children living in southern Spain had fatty tissues, placenta, umbilical cord serum and human milk examined to assess the distribution of endosulfan and metabolites in fatty and non-fatty tissues and fluids (Cerrillo et al., 2005). It was shown in previous studies that endosulfan is transmitted from mother to child via milk (Campoy et al., 2001) and that endosulfan residues were found in 40% and 30% of adipose tissue samples from children living in Murcia and Granada (Southern Spain), respectively (Olea et al., 1999). This transfer from milk to child raises a concern regarding a potential source of endosulfan exposure to infants and children.

*3.2.5.3. Fetuses.* It was proposed that pesticide exposure (including endosulfan and dicofol) to pregnant women living near agricultural applications induced neurotoxicity in fetuses when exposure occurred during gestation weeks 1 through 8 (period of central nervous embryogenesis) (Roberts et al., 2007). Exposure to unknown levels of endosulfan and dicofol was proposed to result in an increased incidence in autism spectrum disorder (ASD). The study authors cautioned that there were many flaws to the study, no knowledge of diet, actual duration of exposure, if the selected population was actually exposed to these pesticides and possible misclassification of both the exposure and outcome (see Christensen, 2007 and Erdreich and Morimoto, 2007 for detailed analyses of Roberts et al., 2007) and there is no "cause and effect" between endosulfan and ASD. Currently the cause of ASD is not known. Indications were that an association was not determined but that the hypothesis "requires further study."

*3.2.6. Genotoxicity and oncogenicity*

Flodstrom et al. (1988) used endosulfan (α-,β-isomers separately and α-,β-isomer ratio 70:30) and metabolites (endosulfan sulfate, alcohol, ether and lactone) *in vivo* and *in vitro* to assess carcinogenic potency (ability to enhance enzyme altered foci (EAF) in rat liver), tumor promoting ability and inhibition of intercellular communication. EAF were not induced *in vivo* after endosulfan treatment at 1 and 5 mg/kg/day for 10 weeks. An *in vitro* Chinese hamster lung fibroblast (V79) metabolic cooperation assay and a scrape loading/dye transfer assay with rat liver WB epithelial cells were also performed to assess intercellular communication after endosulfan treatment. Results showed *in vitro* at non-cytotoxic doses endosulfan (α- or β-separately; α- + β-mixture; endosulfan sulfate) inhibited gap junction communication in both assays. Hepatocyte gap junctional intercellular communication was also inhibited by endosulfan metabolites (sulfate, lactone, ether) *in vitro* (Ruch et al., 1990). Authors concluded "the highest dose used in the EAF study with endosulfan was 5 mg/kg/day since higher doses increased mortality in a pilot study. This high systemic toxicity could possibly preclude the detection of a tumor promoting activity requiring higher tissue concentrations to operate. . .alternatively, the *in vitro* systems used in this study may have produced false-positive results with respect to the promoting potential of endosulfan" (Flodstrom et al., 1988).

Two rat *in vivo* tumor promotion EAF studies yielded one positive (Fransson-Steen et al., 1992) and one negative result (Fransson-Steen and Warngard, 1992). The positive study provided evidence that α-endosulfan (but not β- or α- + β-) serves as a tumor promoter in rats with two-thirds hepatectomy at highly toxic doses (15 mg/kg/day) that also induce systemic and/or neurotoxic effects. In primary rat hepatocytes, β-endosulfan is a more potent inhibitor of intercellular communication than α-endosulfan. However, the two isomers had similar inhibitory potency in WB-Fischer 344 rat liver epithelial cells (Fransson-Steen and Warngard, 1992).

For genotoxicity, numerous studies have been performed in bacteria, yeast, mammalian cells in culture and *in vivo* in laboratory animals (Adams, 1978; Arnold, 1972; Bajpayee et al., 2006; Chaudhuri et al., 1999; Cifone, 1983, 1984a,b; Daniel et al., 1986; Dikshith and Datta, 1977; Dikshith et al., 1978; Dorough et al., 1978; Dubois et al., 1996; Dzwonkowska and Hubner, 1986; Fahrig, 1974; Kurinnyi et al., 1982; Lu et al., 2000; L'vova, 1984; McGregor et al., 1988; Mellano, 1984; Milone and Hirsch, 1986; Moriya et al., 1983; NTP, 2007; Pednekar et al., 1987; Quinto et al., 1981; Sharma and Gautam, 1991; Shirasu et al., 1978, 1982; Sobti et al., 1983; Usha Rani et al., 1980; Usha Rani and Reddy, 1986; Velazquez et al., 1984; Yadav et al., 1982). There is evidence of genotoxicity in tests for gene mutation, chromosomal aberration and DNA damage in open literature studies, however other tests for these effects (i.e. all FIFRA Guideline studies, as well as open literature studies) are negative (described and summarized in Silva, 2008). Since there are well-conducted studies that are positive, endosulfan is considered to be genotoxic.

When considering the results of all available *in vivo* studies performed in rats and mice, there is insufficient evidence indicating endosulfan is oncogenic in the studies conducted to date. There were acceptable studies with well designed, peer reviewed protocols performed in rat (104 week chronic/oncogenicity) and in mouse (18 month) that resulted in no indication that endosulfan is oncogenic. The NOEL for mice in an acceptable FIFRA Guideline oncogenicity study was 0.84 mg/kg/day (M) and 0.98 (F) mg/kg/day, based on mortality (Donaubauer, 1988). There were no tumors that were treatment-related, dose-related or otherwise different in incidence across dose groups. Therefore, endosulfan was not considered to induce tumors in mice after "lifetime" treatment.

Endosulfan is categorized as "A4" (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH, 2007). USEPA Health Effects Division (Cancer Peer Review Committee) stated that there was no evidence of carcinogenicity for endosulfan. Endosulfan was placed in Group E: Evidence of non-carcinogenicity for humans (USEPA, 2007b). The Canadian Preliminary Risk and Values Assessment for Endosulfan states "Endosulfan was not carcinogenic in mice or rats and was not genotoxic," (Health Canada, 2007). The World Health Organization also considered endosulfan to be non-carcinogenic (WHO, 2006).

**Table 4**
Comparison of critical NOEL/NOAEL and endpoints for risk characterization: CDPR and USEPA.

| Exposure | 1. CDPR NOEL mg/kg/day | 2. USEPA NOAEL mg/kg/day | Effects/endpoints (References) |
|---|---|---|---|
| Acute oral/dermal: general population, including infants and children | Both oral and dermal NOEL = 0.7 developmental, rabbit | Oral NOAEL = 1.5[c], neurotoxicity, rat<br>Dermal NOAEL = 1.25[d] (estimated) DNT, rat | 1. Abortions, death, convulsions, neurotoxicity post-dose[(1)]<br>2. ↑ Convulsions (F) 8 h post-dose [(2)] |
| Acute inhalation: (1–30 d) and subchronic (1–6 mos)[a] | Inhalation NOEL = 0.194[a] 21 d Inhalation, rat | Inhalation NOEL = 0.2[c] 21 d Inhalation, rat | 1. and 2. ↓ Body weight gain and lymphocyte counts (M); ↑ creatinine values (F)[(3)] |
| Subchronic oral/dermal:short (1–30 d) and intermediate (1–6 mos) | Both oral and dermal<br>NOEL = 1.18 Reproduction, rat 47.3% Dermal absorption | Dermal NOAEL = 1.25 (estimated) DNT, rat 45% Dermal absorption | 1. ↑ Kidney and liver weights; ↓ food consumption and body weight gains[(4)]<br>2. ↓ Pup weight[(5)] |
| Chronic oral/dermal: general population, including infants and children | Both oral and dermal NOEL = 0.57 Dietary, dog (1 year) | Oral NOEL 0.6 mg/kg/day[c] Chronic/oncogenicity, rat Long-term dermal exposure is not expected | 1. Premature deaths, neurotoxicity; ↓ body wt gain and food consumption[(6)]<br>2. ↓ Body wt gain, ↑ marked progressive glomerulonephrosis and blood vessel aneurysms (M)[(7)] |
| Chronic inhalation (>6 months) | Inhalation estimated NOEL = 0.0194[b] 21 d Inhalation, rat | Long-term inhalation exposure is not expected | 1. ↓ Body wt gain and lymphocyte counts in (M); ↑ creatinine (F)[(3)] |
| Cancer | Group E-evidence of non-carcinogenicity for humans | | Q* not calculated. Chronic combined study (rats) and carcinogenicity study (mice) resulted in no ↑ frequency of tumors |

*References:* 1. Nye (1981); 2. Bury (1997); 3. Hollander et al. (1984); 4. Edwards et al. (1984); 5. Gilmore et al. (2006); 6. Brunk (1989); 7. Ruckman et al. (1989).
[a] The subchronic inhalation NOEL of 0.194 mg/kg/day was used by CDPR for both the acute and subchronic NOEL.
[b] A 10× UF is added to the subchronic inhalation NOEL to extrapolate to obtain a chronic inhalation "estimated NOEL" = (subchronic inhalation NOEL) ÷ UF 10× extrapolation from subchronic to chronic exposure (USEPA, 2004).
[c] Values also used in the preliminary risk assessment for endosulfan by Health Canada (2007).
[d] Estimated by 1/3rd LOAEL (3.74 mg/kg/day).

## 4. Discussion of endpoints for CDPR and USEPA (summary in Table 4)

In all cases (acute, subchronic and chronic) CDPR and USEPA used oral (gavage or dietary) studies as surrogates for dermal studies. Route-to-route extrapolation is an acceptable method for endosulfan since the toxicity does not differ between oral and dermal treatment (USEPA, 2004). In addition, dermal penetration factors based on Craine (1988) were used (CDPR = 47.3%; USEPA = 45%).

### 4.1. Acute toxicity (Table 4)

#### 4.1.1. Oral

CDPR selected the critical acute oral (gavage) NOEL from a rabbit developmental toxicity study (Nye, 1981) because the pregnant dams showed clinical signs of neurotoxicity the first day of gavage treatment and therefore the effects were acute. Pregnant dams and fetuses represented a potentially sensitive population group. The NOEL of 0.7 mg/kg/day was lower than the NOEL/NOAEL of 1.5 mg/kg/day obtained in the acute oral rat neurobehavioral toxicity test (performed in adult animals) selected by USEPA (Bury, 1997; USEPA, 2007b). Uncertainty was generated by the fact that the rabbit developmental study involved multiple dosing, but because neurotoxicity occurred on the first day of dosing, subsequent treatment did not appear to be additive. CDPR concluded that pregnant rabbits were more responsive to low gavage doses of endosulfan than non-pregnant female or male rats. The USEPA NOAEL acute oral selection (1.5 mg/kg/day) was twice as high as that of CDPR. The study was specifically designed to detect neurobehavioral toxicity after oral treatment and USEPA selected this study to use in their acute dietary risk characterization estimates (USEPA, 2002, 2007b).

#### 4.1.2. Dermal

CDPR used the oral NOEL (0.7 mg/kg/day) from the rabbit developmental study (Nye, 1981) as a surrogate for a dermal study. This low NOEL was considered health protective when used to estimate dermal MOEs for all occupational exposures, including those to female workers and for children exposed dermally as swimmers in surface water (Beauvais et al., 2009a,b; Silva, 2008). The USEPA selected the rat dietary DNT study for their lowest critical "estimated" NOAEL (1.25 mg/kg/day), based on dam and pup weight loss (see Section 3.2.1.3 *USEPA Acute "Dermal"*, above). This endpoint was considered by the USEPA to be sufficient to protect the occupational population considered most at risk (female workers).

#### 4.1.3. Inhalation

Inhalation exposure scenarios for bystanders at pesticide application sites are presented for infants and adults in the CDPR risk assessment (Beauvais et al., 2009b). There were several CDPR acute inhalation-specific scenarios both occupationally and to the general population where a critical acute NOEL was necessary to complete the risk assessment for endosulfan (Silva, 2008).

While the CDPR selection of the subchronic NOEL (0.194 mg/kg/day) for the acute NOEL involved some uncertainty, the advantages to this method rather than extrapolating from the $LC_{50}$ are: (1) LOELs (LDT) from the acute, rangefinding and subchronic studies were similar (0.61, 0.44 and 0.387 mg/kg/day), (2) more animals treated in the subchronic (15/sex/dose subchronic vs. 5/sex/dose acute), (3) the subchronic had a 29 day recovery vs. acute with a 14 d observation; (4) the estimate is low for an acute NOEL, since acute NOELs are usually higher than subchronic. All three studies were performed at the same laboratory and in the same time frame (1983). CDPR used this NOEL to estimate MOEs for occupational and general population scenarios (infant and adult bystanders at application sites). The subchronic inhalation study (NOAEL = 0.2 mg/kg/day; 0.001 mg/L; Hollander et al., 1984) was also used by USEPA for their short-term (1–30 day) occupational exposure MOE estimates (USEPA, 2007b).

### 4.2. Subchronic toxicity (Table 4)

#### 4.2.1. Oral/dermal

The critical oral subchronic NOEL of 1.18 mg/kg/day from the rat reproduction study was used by CDPR for dietary and

"non-dietary ingested" by swimmers in surface water MOE estimates, as well as dermal (occupational; swimmers in surface water) MOE estimates (Beauvais et al., 2009a,b; Edwards et al., 1984). Besides the low NOEL, an advantage to selecting the reproduction study was observing the results of the exposure to potentially more at risk populations (rat fetuses, neonates, weanlings), compared with adult animals. The 90-day subchronic rat dietary NOEL was higher (1.92 mg/kg/day) but both studies had the common endpoints of increased kidney and liver weights (Barnard et al., 1985; CDPR, 2008).

The USEPA evaluations resulted in selection of the DNT study (Gilmore et al., 2006) for the critical subchronic oral/dermal NOAEL for dermal occupational MOE determinations and dietary risk characterization. In the 2002 RED, USEPA chose the two 21-day dermal toxicity studies performed in rat and used them as the basis of quantifying dermal occupational risk (Ebert et al., 1985a,b). The dermal NOAEL was 12 mg/kg/day based on mortality. The DNT study examined parameters in neonates, pups and weanlings that were not evaluated in the 21-day dermal study (conducted in adult animals only). USEPA considered that the use of an offspring endpoint from the DNT study would be the appropriate endpoint to protect female workers. This decision is supported by the pup weight decrements that were observed only during lactation (i.e., the pup body weights were not affected at birth and the pups recovered at PND 22) and therefore are likely due to nursing. The USEPA extrapolated from a LOAEL to NOAEL (LOAEL = 3.74 mg/kg/day) as previously described, using a 3× UF based on pup weight loss in the DNT and in the 2-generation reproduction study (USEPA, 2007b).

The rat dietary subchronic neurotoxicity study, required by USEPA in the 2002 RED (USEPA, 2002) reported no neurobehavioral or neuropathological effects, nor were there effects on reproductive organs, or endocrine organs in either sex (Sheets et al., 2004). The study was not selected as a definitive study because the NOEL (2.11 mg/kg/day) was higher than the critical oral/dermal NOEL selected by CDPR and USEPA.

#### 4.2.2. Inhalation

See Section 4.1.3 above: inhalation.

### 4.3. Chronic toxicity (Table 4)

#### 4.3.1. Oral/dermal

A source of uncertainty in the chronic dog study selected by CDPR was the incomplete characterization of the neurotoxic effects (Brunk, 1989). This did not, however, affect the NOEL. Due to the dosing schedule, some of the neurotoxic effects (mid and high dose dogs experienced violent contractions in the upper abdomen, in the absence of vomiting) were not discovered until day 136 of treatment. The effects didn't occur until 2.5–6 h post-dosing, and when dosing occurred in the afternoon, they were missed. When discovered, the dosing schedule was changed for the rest of the study period, but it is not known when these effects initially occurred, or if they had changed over time. The dogs showed effects at low doses such that it was necessary to terminate animals early at the high dose (1.95 mg/kg/day), where this did not occur in the rat study where the high dose was even greater (2.9 mg/kg/day). The USEPA selected the rat combined study (Ruckman et al., 1989), with effects in kidney and aneurysms, and a NOAEL of 0.6 mg/kg/day where the dog study had a NOEL of 0.57 mg/kg/day. Both CDPR and USEPA used their NOEL/NOAELs for chronic dietary risk characterization (detailed in Silva and Carr, 2009). CDPR also used their value to estimate annual MOEs for occupational scenarios and for swimmers in surface water (children and adults; non-dietary ingested and dermal) (Beauvais et al., 2009a,b; Silva, 2008; USEPA, 2007b).

#### 4.3.2. Inhalation

CDPR estimated a critical chronic NOEL from the subchronic NOEL (Hollander et al., 1984) with the addition of a 10× UF. This approach adds some uncertainty to the chronic inhalation MOE estimates for both occupational and general public (bystanders at application sites) scenarios (Beauvais et al., 2009a,b; Silva, 2008).

### 4.4. FQPA issue

CDPR is in agreement with the final USEPA decision on the lack of a need for additional safety factors (SF) under the FQPA for endosulfan. The following discussion provides the basis of the initial concerns generated in FIFRA Guideline studies and in the open literature regarding the potential for endosulfan to cause reproductive toxicity in males or to serve as a potential endocrine disruptor. The concerns requiring re-evaluation of FQPA SF were also addressed in the CDPR risk assessment.

#### 4.4.1. Re-evaluation of the FQPA safety factor due to concerns generated from the FIFRA guideline studies (Table 5)

The USEPA re-evaluated the status of the FQPA SF and changed it from 10× to 1× based on the results of the DNT study and the weight of evidence of all studies submitted by the registrants (USEPA, 2007b). In the DNT study (Gilmore et al., 2006), the USEPA required that the evaluation of sperm count, motility and morphology in young adult male rats since sperm parameters were not previously required and had not been examined in the 2-generation reproductive toxicity study (Edwards et al., 1984). In the DNT study, no effects were observed in sperm count, sperm mobility or sperm morphology at any dose but there was a possible delay in PPS at 29.8 mg/kg/day. Pup body weights at 29.8 mg/kg/day (HDT) were decreased by 10% at PND 21 ($p < 0.01$), and remained statistically significantly decreased through PND 75 at HDT. The delayed PPS was likely secondary to pup toxicity experienced at 29.8 mg/kg/day.

In the 104-week chronic rat study (Ruckman et al., 1989), absolute testis weight appeared to decrease at 2.9 mg/kg/day (HDT). The testis weights and body weights were within historical control range and therefore the observation may have been incidental and not toxicologically relevant. The NOEL for this study was 0.6 mg/kg/day, and would be protective for any effects occurring at the higher doses. There were no effects on testis in any of the other FIFRA Guideline studies reviewed.

Pituitary and uterine weight effects were observed in the dietary 2-generation rat reproductive toxicity study. The increased relative pituitary weights in F1a pups (not F1b pups) and the increased uterine weights in F2a pups (not F2b) were seen only at the HDT (6.2 mg/kg/day). This is 5 times higher than the subchronic NOEL selected by CDPR (1.18 mg/kg/day) and more than 10 times higher than the chronic NOEL selected by CDPR and USEPA (dog = 0.57 and rat = 0.6 mg/kg/day, respectively). Therefore the questionable effects in pituitary and uterus are considered of limited toxicological relevance for the purpose of risk assessment.

#### 4.4.2. Re-evaluation of the FQPA safety factor due to concerns generated from open literature reports on male rat reproductive endosulfan-induced toxicity (Table 5)

The results of Sinha et al. (1995, 1997 and 2001) generated concerns due to effects to the reproductive systems of males, however the data were not useful for the purpose of risk assessment because of several major flaws associated with study designs and methods. These flaws included the use of Druckrey rats instead of a well-characterized strain like Wistar or Sprague–Dawley rats, the lack of consistency among studies for sperm parameters, the lack of historical controls for Druckrey rats, the use of small test groups (3–5 rats), no clinical observations, no individual data, positive controls or concurrent comparison groups for adults and

**Table 5**
Reproduction effects in young males vs. adults (M/F) after endosulfan exposure.

| References[a] | Animal strain | Treatment duration | #/dose | LOEL/NOEL (mg/kg/day)[b] |
|---|---|---|---|---|
| *Effects in rats* | | | | |
| 1[c] | Druckrey | 90 d old M treated 70 d | 5 M | Male LOEL = 2.5 |
| 2[c] | Druckrey | 3 weeks old M pups; treated 90 d, 5 d/weeks | 5 M | Male LOEL = 2.5 |
| 3[c] | Druckrey | Treat GD 12 to birth<br>Cross-fostered pups to PND 100 | 3 dams | Male LOEL = 1.0 |
| 4 | Wistar | Adult M gavage 30 d prior to mating | 5 M/10F | **Reproductive NOEL > 5.0 M; 1.5 F**<br>**Systemic NOEL = 2.5 M; 0.75 F** |
| 5[c] | Wistar | Dams treated GD 15–LD22<br>F1 observed PND 65 and 140<br>Mate F1 M to virgin F PND 120<br>F2 observations on PND 21 | 8 dams F1<br>8 litters F2 | **Male reproductive NOEL > 1.5**<br>**Male systemic NOEL = 1.5** |
| 6[c] | Wistar | Dams treated 21 d premating to pup weaning<br>F1 observations PND140 | 8 M | **Dam and M pup reproductive NOEL > 1.5**<br>**Dam and M pup systemic NOEL > 1.5** |
| 7 | Wistar | Dams treated GD 1 through PND 28 | 10 M | **Dam and M pup reproductive NOEL > 2.5**<br>**Dam systemic NOEL = 1.0** |
| 8[d] | Wistar Crl:WI(Han) | Dams treated GD 6 through LD 21<br>F1 observations PND 21, PND 75 | 23 litters; 4 M/litter | **Dam and M pup reproductive NOEL > 29.8**<br>**Dam and M pup systemic LOEL = 3.74 (LDT)** |
| 9[d] | Crl:COBS (CD) BR | 2 Generation; 2 litters/generation | 28–30 M/F | **Dam and pup reproductive NOEL > 5.9**<br>**M/F adult and pup systemic NOEL = 1.18** |
| 10[d] | CD SD | Dams treated GD 6 through 19 | 28 dams | **Fetal developmental NOEL = 2.0**<br>**Dam systemic NOEL = 2.0** |
| *Effects in rabbits* | | | | |
| 11[d] | New Zealand White | Dams treated GD 6 through 28 | 26 dams | **Fetal developmental NOEL > 1.8**<br>**Dam systemic NOEL = 0.7** |

*Abbreviations:* LD, lactation day; GD, gestation day; PND, post-natal day; M, male; F, female; LDT, lowest dose tested; HDT, highest dose tested.
Bold, studies where NOELs for reproduction or developmental effects exceed the HDT and/or systemic NOEL.
[a] 1. Sinha et al. (1995); 2. Sinha et al. (1997); 3. Sinha et al. (2001); 4. Dikshith et al. (1984); 5. Dalsenter et al. (1999); 6. Dalsenter et al. (2003); 7. Zhu et al. (2000); 8. Gilmore et al. (2006); 9. Edwards et al. (1984); 10. Fung (1980a,b); 11. Nye (1981). Also see Silva and Gammon (2009) for a complete discussion of the reproductive effects related to endosulfan.
[b] Reproductive effects may include: sperm count, sperm production, sperm motility, sperm morphology, testes weights, epididymal weights, fertility, mating, litter or offspring effects.
[c] Studies where clinical observations for neurotoxicity were not reported.
[d] Indicates FIFRA Guideline studies.

pups. When sample sizes are small for parameters such as sperm count (where the potential for broad variation occurs) and studies are not repeated, then it is problematic to rely on these studies for risk assessment. This is especially true if the effects in question did not appear in other, well-conducted studies.

#### *4.4.3. Studies examining the possibility of increased sensitivity to endocrine disruption in males after treatment with endosulfan (Table 5)*

Dalsenter et al. (1999) investigated the effects of endosulfan on Wistar F1 male fetuses/neonates after gavage treatment of dams GD 12 through PND 22 at 0 (Tween 80), 1.5 and 3.0 mg/kg/day. F1 males were evaluated for reproductive effects on PND 65 and 140. Some F1 males were mated on PND 120 to assess effects on fertility and reproduction and to evaluate effects on F2 litters. At 3.0 mg/kg/day effects on male offspring appeared to be toxicologically significant and possibly related to maternal toxicity. The males mated on PND 120 to untreated females resulted in no reproductive effects in the dams or offspring. It can be concluded that treatment of dams GD 12 to PND 22 provides a NOEL of 1.5 mg/kg/day for reproductive effects in adult males and treated dams.

Dalsenter et al. (2003) treated dams by gavage with endosulfan at 0 (sunflower oil), 0.5 and 1.5 mg/kg/day from 21 days prior to mating through lactation in order to test the effect of endosulfan on male offspring. No maternal toxicity was observed and there were no effects on male reproductive parameters at 1.5 mg/kg/day (HDT) when male pups were evaluated up to PND 140. These data support the previous results of Dalsenter et al., 1999 as well as the 2-generation reproductive toxicity study of Edwards et al., 1984; NOEL = 1.18 mg/kg/day.

Zhu et al. (2000) administered endosulfan by gavage to pregnant Wistar rats from GD 0 through PND 28 to investigate the effect on fetuses and pups at 0 (vegetable oil), 0.5, 1.0 and 2.5 mg/kg/day. The NOEL is 1.0 mg/kg/day (comparable to the rat reproduction NOEL of 1.18 mg/kg/day) based on systemic effects (neurotoxicity, death, decreased body weight) and not developmental or reproductive effects. In the dietary DNT study (FIFRA Guideline: USEPA, 2007a; Gilmore et al., 2006) there were no effects on Wistar male pups on reproduction parameters at any dose up to PND 75. Dikshith et al., 1984 showed that males treated with endosulfan via gavage for 30 days prior to mating had no reproductive effects at doses of 5.0 mg/kg/day. There were also no effects to the F1 litters of the treated adult males. Neurotoxicity was observed in dams at 0.75 mg/kg/day and in males at 2.5 mg/kg/day. Fung (1980a,b) showed that rat pups were affected with visceral and skeletal anomalies at doses that were either identical to or greater than those causing effects in dams (NOEL = 2.0 mg/kg/day). This was also observed with rabbits (NOEL = 0.7 mg/kg/day; Fung, 1981a,b; Nye, 1981).

Although many of the open literature reports have deficiencies, they support the findings of the DNT study and the 2-generation rat reproductive toxicity study where the NOELs were based on body weight reductions and other systemic effects in adults and pups (see Silva and Gammon, 2009 for a review of the developmental, reproductive and neurotoxic effects related to endosulfan). Results did not indicate that rat or rabbit offspring are more susceptible to endosulfan effects than are adults. Therefore, USEPA and CDPR are in agreement that additional SF for potential increased pre- and post-natal sensitivity (endocrine disruption) is not necessary.

## 5. Conclusions

### *5.1. NOELs*

The acute oral NOEL of 0.7 mg/kg/day (Nye, 1981) was selected by CDPR, since pregnant rabbit was considered to be more responsive to low gavage doses of endosulfan than non-pregnant female

or male rats. USEPA selected an acute oral NOAEL of 1.5 mg/kg/day (Bury, 1997) from a study that was specifically designed to detect neurobehavioral toxicity in adult rats after acute oral treatment. The acute oral NOEL/NOAELs were the only ones that differed between CDPR and USEPA, and both were sufficiently low to necessitate risk assessment. The NOEL/NOAELs for other exposure durations, both oral and inhalation were similar between CDPR and USEPA (Subchronic: CDPR = 1.18 mg/kg/day [Edwards et al., 1984]; USEPA ~1.25 mg/kg/day [estimated; Gilmore et al., 2006]; Chronic: CDPR = 0.57 mg/kg/day [Brunk, 1989]; USEPA = 0.6 mg/kg/day [Ruckman et al., 1989]; acute/subchronic inhalation: CDPR and USEPA = 0.001 mg/L; CDPR chronic inhalation = 0.0001 mg/L [estimated]; Hollander et al., 1984). CDPR and USEPA used oral (gavage or dietary) studies as surrogates for dermal studies since the toxicity did not differ between these routes (USEPA, 2004). Dermal penetration factors based on Craine (1988) were used (CDPR = 47.3%; USEPA = 45%).

The NOEL/NOAELs selected by both agencies in all exposure intervals were very low and this will affect the MOEs as will be shown in accompanying publications in this issue (dietary risk assessment: Silva and Carr, 2009; occupational and public risk: Beauvais et al., 2009a,b). The NOELs generated from this hazard ID will be used to calculate the MOEs and characterize risk for all exposure scenarios for endosulfan.

### 5.2. Uncertainty

The acute oral NOEL selected by CDPR (rabbit developmental study; Nye, 1981) involved multiple dosing, but because neurotoxicity occurred on the first day of dosing, subsequent treatment did not appear to be additive. The chronic oral NOEL selected by CDPR (1 year dog; Brunk, 1989) had an incomplete characterization of the neurotoxic effects early in treatment. This did not, however, affect the NOEL. While there were uncertainties in each study, the low NOELs are health protective for these exposure durations.

CDPR selected a subchronic inhalation NOEL (0.194 mg/kg/day; Hollander et al., 1984) to use for an acute NOEL. This decision was based on similar acute neurotoxicity (clinical signs) observed in three studies ($LC_{50}$ [Hollander and Weigand, 1983], subchronic rangefinding and definitive study [Hollander et al., 1984]) with similar LOEL values. In general an acute NOEL is higher than a subchronic NOEL, so in that respect, the use of the subchronic NOEL is more health protective. A chronic inhalation study was not available so a 10× UF was introduced to extrapolate from a subchronic to a chronic NOEL (Hollander et al., 1984; USEPA, 2004). While uncertainty was introduced, the resulting NOEL was very low and therefore potentially more health protective.

### 5.3. FQPA

Endosulfan has been shown to induce severe neurotoxicity as its primary effect and the mechanism of action for endosulfan is well described (Lawrence and Casida, 1984; Cole and Casida, 1986). However, other endpoint concerns (e.g. reproductive or developmental toxicity) were addressed by both CDPR and USEPA and the over-riding effects were systemic (e.g. body weight or neurotoxicity) occurring at doses lower than those inducing other toxicity. A primary risk assessment concern for endosulfan is that effects resulting from exposure during critical developmental stages (*in utero*, or to infants and children) will result in endocrine disruption and subsequent neurotoxicity, developmental or reproductive adverse effects that are irreversible. These concerns were addressed primarily with the DNT study required of registrants under the FQPA (1996). The FQPA SF was reduced from 10× to 1× by the USEPA after review of the DNT study and analysis of the endosulfan database (USEPA, 2007b). Both CDPR and USEPA agree that data do not support the case that endosulfan is a developmental or reproductive toxicant or an endocrine disruptor at doses lower than those that cause other toxic effects.

## Acknowledgments

From CDPR, we thank Joyce Gee, Lori Lim and Carolyn Lewis and from USEPA, we thank Tracy Perry, Donald Wilbur, Elissa Reaves and Judy Facey for discussions and/or writings that were drawn upon for this article.

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