Office of Environmental Health Hazard Assessment

Joan E. Denton, Ph.D., Director
Headquarters • 1001 I Street • Sacramento, California 95814
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010
Oakland Office • Mailing Address: 1515 Clay Street, 16th Floor • Oakland, California 94612

Linda S. Adams
Secretary of Environmental Protection

Arnold Schwarzenegger
Governor

MEMORANDUM

TO: Gary T. Patterson, Ph.D., Chief
Medical Toxicology Branch
Department of Pesticide Regulation

Sue Edmiston, Chief
Worker Health and Safety Branch
Department of Pesticide Regulation

FROM: Anna M. Fan, Ph.D., Chief
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

Melanie Marty, Ph.D., Chief
Air Toxicology and Epidemiology Branch
Office of Environmental Health Hazard Assessment

DATE: June 4, 2008

SUBJECT: REVISED FINDINGS ON THE HEALTH EFFECTS OF THE ACTIVE INGREDIENT: ENDOSULFAN

Enclosed please find a copy of the revised final Office of Environmental Health Hazard Assessment's (OEHHA) findings for the active ingredient endosulfan. These findings contain one minor change to item #20, after consultation with Tobi Jones. The middle sentence of that finding has been reworded to: "...When using NOAELs from animal studies, DPR considers MOEs of greater than 100 to be health protective, regardless of the route of exposure."

Should you have any questions Dr. David Ting at (510) 622-3226, or Dr. Anna M. Fan at (510) 622-3165.

Enclosure

cc: (next page)

cc: Allan Hirsch
Chief Deputy Director
Office of Environmental Health Hazard Assessment

George V. Alexeeff, Ph.D., D.A.B.T.
Deputy Director for Scientific Affairs
Office of Environmental Health Hazard Assessment

David Ting, Ph.D., Chief
Pesticide and Food Toxicology Section
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

Charles Vidair, Ph.D.
Staff Toxicologist
Pesticide and Food Toxicology Section
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

John Budroe, Ph.D.
Staff Toxicologist
Air Toxicology and Epidemiology Branch
Office of Environmental Health Hazard Assessment

Jim Behrmann
Liaison, Scientific Review Panel
Air Resources Control Board

Office of Environmental Health Hazard Assessment's Findings On the Health Effects of Endosulfan

Pursuant to Food and Agricultural Code Sections 14022 and 14023, the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency (Cal/EPA) provides consultation and technical assistance to the Department of Pesticide Regulation (DPR) on the evaluation of health effects of candidate toxic air contaminants (TAC) and prepares health-based findings. OEHHA previously reviewed and commented on the draft documents prepared by DPR on the evaluation of human health risks associated with potential exposure to endosulfan. These documents are used by DPR in considering whether to list endosulfan as a TAC. As part of its statutory responsibility, OEHHA has also prepared these findings on the health effects of endosulfan that are to be included as part of DPR's Risk Characterization/ Toxic Air Contaminant (RCD/TAC) documents.

Chemical Identification

1. Endosulfan is an insecticide used to kill a wide variety of insects infesting a range of crops. It is classified as a chlorinated hydrocarbon of the cyclodiene group, or organochlorine. Endosulfan exists in α and β isomeric forms. The α isomer is a more potent inhibitor of chloride flux in nerve cells (see Mechanisms of Toxicity below) and has been found at higher concentrations in air monitoring studies (see below).

Usage and Reported Illnesses

2. Today the crops most commonly treated with endosulfan are grapes, melons, lettuce, tomatoes and cotton. Currently there are six formulated products containing endosulfan that are registered in California. The yearly use of endosulfan in California has been declining, from 180,000 pounds in 1998 to 153,000 pounds in 2004.

3. Between 1992 and 2005, the Pesticide Illness Surveillance Program of DPR recorded 58 illnesses that likely involved exposure to endosulfan. Of these, four resulted from drift in the air following endosulfan application. Most of the illnesses were skin and/or eye irritation. It was not indicated in the Volume II Exposure Assessment document how many of these illnesses were non-occupational.

Environmental Fate

4. Endosulfan in the environment is subject to both hydrolysis and photolysis. Fungi and bacteria degrade endosulfan under both aerobic and anaerobic conditions. Endosulfan adsorbs strongly to soil. California drinking water systems drawing their water from surface water bodies or from wells were monitored for endosulfan from 1986 to 2003. The absence of endosulfan from surface-derived

samples, and the low percentage of positive samples from well water, suggest that drinking water is not a significant source of human exposure to endosulfan.

5. Air monitoring in California shows that endosulfan can drift many miles after aerial application to field crops. It also volatilizes from soil, water and plants. Thus, populations close to or far from agricultural fields can be exposed via the air.

6. Endosulfan bioaccumulates in aquatic plants and animals. It is rapidly cleared from aquatic animals post-exposure.

Endosulfan in Ambient Air

7. The ambient air is defined as the air away from agricultural sites of endosulfan application. Endosulfan has been detected in ambient air sampled from urban and unpopulated areas in three studies of agricultural applications in California in 1985, 1996 and 1999. In 1985 DPR monitored the air at three residential sites near agricultural fields in Monterey County. In 1996 the California Air Resources Board (ARB) sampled air in Fresno County over a five-week interval during the summer; these included four monitoring sites located in populated areas in the vicinities of agricultural land and one urban site. In 1999 the ambient air was also monitored in Tulare County in a study designed to determine if endosulfan moved up-slope into the Sierras as a result of its application in the Central Valley. The 1996 ARB study is discussed here because it contained the greatest number of endosulfan detections, and the levels were higher than those of the other two studies. The monitoring period (July 29 to August 29) approximately corresponded to the period of greatest endosulfan use (June-August). Air samplers were placed approximately 1.5 meters above single-story school buildings in vicinities of agricultural fields. The sampler at the urban site was placed above a two-story building. For α-endosulfan, 66 of 75 samples taken from the sites near agricultural land contained concentrations above the limit of quantification (LOQ, determined by the analytical limit of detection and quantity of air sampled), ranging from 0.0095 to 0.32 $\mu g/m^3$. For β-endosulfan, only two of 75 samples (0.016 and 0.031 $\mu g/m^3$) were above the LOQ. None of the samples from the urban site had endosulfan levels above the LOQ.

Endosulfan in Air Near Application Sites (For Calculating Bystander Exposures)

8. Persons near pesticide application sites are subject to relatively high exposures via inhalation should the chemical drift in the air into the area immediately surrounding the field (termed bystander exposure). The ARB monitored endosulfan concentrations near an apple orchard treated by airblast application of endosulfan in San Joaquin County in 1997. Four monitoring sites surrounded the orchard within approximately ten meters of an edge. Application occurred on April 8 between 5:45 and 7:45 am. Air sampling was for 26.75 and 74.5 hours, starting at the time of application. For 28 samples, 27 α-endosulfan

concentrations were above the LOQ, ranging from 0.004 to 4.56 $\mu g/m^3$. For β-endosulfan, 16 samples had concentrations above the LOQ, ranging from 0.012 to 0.34 $\mu g/m^3$. A 24-hour time-weighted average (TWA) concentration for the day of application, and a 3-day TWA concentration that included the two days post-application are estimated in the Volume II Exposure Assessment document. The TWA concentrations of endosulfan were used to calculate short-term (24-hour TWA), seasonal (3-day TWA) and annual (3-day TWA) bystander exposures. These bystander exposures were anticipated to equal or exceed any exposures that might occur at locations away from the application site. Therefore, human exposure via the ambient air (away from the application site) was not calculated. OEHHA agrees with this approach.

Calculating Bystander Exposures

9. Short-term (up to one week), seasonal (one week to one year) and annual (approximately one year) bystander exposures (Table 1) are estimated in the Volume II Exposure Assessment document. For monitoring performed near an apple orchard treated by airblast application of endosulfan (see above), the monitoring station with the highest measured values gave a 24-hr TWA of 1.63 $\mu g/m^3$ and a 3-day TWA of 0.952 $\mu g/m^3$. The 24-hr TWA was adjusted upward because an application rate of 1.5 lbs of active ingredient per acre (AI/acre) was used instead of the maximum application rate allowed of 2.5 lbs AI/acre. Therefore, the 24-hr TWA was multiplied by 2.5/1.5 to yield 2.72 $\mu g/m^3$. Seasonal and annual exposure estimates were not adjusted in this manner. Breathing rates were 0.59 m^3/kg-day for infants and 0.28 m^3/kg-day for adults. In the absence of data, inhalation absorption was assumed to be 100 percent. Short-Term Absorbed Daily Dosage (STADD) was calculated by multiplying the adjusted 24-hr TWA (2.72 $\mu g/m^3$) by the breathing rate. Seasonal Absorbed Daily Dosage (SADD) was calculated by multiplying the 3-day TWA (0.952 $\mu g/m^3$) by the breathing rate. Annual Absorbed Daily Dosage (AADD) was calculated by dividing the SADD by 12, since it was considered unlikely that repeated applications of endosulfan would occur near the same individual for longer than one month. OEHHA agrees with this approach.

Table 1. Short Term, Seasonal and Annual Bystander Exposures to Endosulfan

	Infants	Adults
STADD mg/kg-day	0.00160	0.00076
SADD mg/kg-day	0.00056	0.00027
AADD mg/kg-day	0.000047	0.000022

Mechanisms of Toxicity

10. Endosulfan binds to the γ-amino-butyric acid (GABA)-gated chloride channel receptor, thereby inhibiting chloride flux. This is thought to be the primary mechanism by which endosulfan causes generalized brain stimulation and neurotoxicity in mammals. Effects of endosulfan on developing male

reproductive organs suggest it is also a developmental toxicant. This may also occur through inhibition of GABA-gated channels, or possibly through direct binding of endosulfan to endocrine receptors. This latter mechanism is supported by the estrogenic, antiandrogenic and proliferative effects of endosulfan tested in cultured MCF-7 human breast carcinoma cells (Andersen et al., 2002; VanParys et al., 2006 and other studies discussed in the Volume I Health Risk Assessment document). Thus, endosulfan is a potential accelerant of estrogen-dependent tumor growth (e.g., breast cancer); however, we are not aware of any studies that have addressed this possibility.

Pharmacokinetics

11. In bile duct-cannulated male rats, approximately 78 percent (α isomer) or 85 percent (β isomer) of an orally administered dose of endosulfan was absorbed by 48 hours (Dorough *et al.*, 1978). If oral absorption equals or exceeds 80 percent, DPR's policy is to assume 100 percent absorption, as was done in this case. Despite endosulfan's lipophilicity, excretion was at least 87 percent by 120 hours post-dosing, mostly through the feces (Dorough *et al.*, 1978). Shortly after oral administration, endosulfan concentrated in the kidney and liver, where it was metabolized into endosulfan sulfate, lactones and ethers. In toxicity studies, the kidneys and liver were sites of increased organ mass and induction of metabolizing enzymes. The high amount of biliary excretion of endosulfan observed by Dorough *et al.* (1978), the rapid (by two hours post-gavage) accumulation of the chemical in the liver and gastrointestinal tract (Chan *et al.*, 2005, Environ Toxicol **20**: 533-541), and a low level of excretion via the urine (approximately 12-16 percent in both studies), suggest a marked first pass effect in the liver for ingested endosulfan that would not be expected following inhalation. Thus, there is the potential for a significantly higher concentration of endosulfan in the general circulation following inhalation compared to exposure to the same dose level via ingestion. This may explain the lower no-observed-adverse-effect-levels (NOAELs) and lowest-observed-adverse-effect-levels (LOAELs) for inhalation studies of endosulfan compared to studies employing the oral route. Comparing studies in the rat, inhalation LOAELs (Hollander and Weigand, 1983; Hollander *et al.*, 1984) were 5- and 10-fold lower than oral LOAELs (Bury, 1997; Barnard *et al.*, 1985) for acute and subchronic dosing, respectively. Therefore, in calculating reference concentrations (RfCs) for endosulfan, OEHHA would consider these pharmacokinetic data when using oral studies to predict responses following inhalation (see Finding 23). It should be noted that ip injection of endosulfan would also be subject to a first pass through the liver (Lukas *et al.*, 1971, The route of absorption of intraperitoneally administered compounds. J Pharm Exp Therap 178:562-566). Dermal absorption was 47 percent over five days in rats. No pharmacokinetic data were located for inhalation exposures. Therefore, it is appropriate to assume 100 percent absorption via inhalation, as was done in the Volume I Health Risk Assessment document.

Acute Toxicity Studies in Animals

12. The lowest oral LD_{50}s for endosulfan were 7.38 mg/kg in male mice and 9.58 mg/kg in female rats (both by gavage). For the oral route, the lowest acute NOAEL was 0.7 mg/kg-day in a rabbit developmental toxicity study (see below) based on clinical signs in does during the first day of treatment. Inhalation LC_{50} values in rats (four-hour exposure) were 34,500 $\mu g/m^3$ (5.52 mg/kg) for males and 12,600 $\mu g/m^3$ (2.02 mg/kg) for females (Hollander and Weigand, 1983). At 3,600 $\mu g/m^3$, where no animals died, the following were observed: dyspnea, trembling, passivity and disturbed equilibrium. At higher concentrations causing some lethality the following were observed: tremors, tonic-clonic convulsions, decreased corneal reflex, decreased papillary light reflex, decreased righting reflex, decreased startle reflex, decreased paw reflex and decreased cutaneous reflex. There was no NOAEL for this acute inhalation study; the LOAEL was 3,600 $\mu g/m^3$ air (0.567 mg/kg) based on the clinical signs described above. The subchronic inhalation study in the rat (see below) however, did identify a NOAEL (0.194 mg/kg-day). This subchronic inhalation NOAEL was lower than the lowest acute oral NOAEL (0.7 mg/kg-day from the rabbit developmental study). Accordingly, OEHHA agrees with the approach followed in the Volume I Health Risk Assessment document, that the most appropriate NOAEL for evaluating acute inhalation exposures in people is the rat subchronic inhalation NOAEL.

Subchronic Toxicity Studies in Animals

13. Over 16 subchronic studies were available, with all but one performed in the rat. Clinical signs of neurotoxicity included tonic/clonic convulsions and behavioral (memory) effects. Pathological effects were most often noted in the liver and kidney and in hematology. For the oral route, the lowest NOAEL was 1.18 mg/kg-day from a rat reproduction study based on increased kidney and liver weights in parental animals treated for 24 weeks. For the inhalation route (Hollander *et al.*, 1984), rats were exposed nose-only for 21 days at six hours per day (five days per week). A NOAEL of 0.194 mg/kg-day (1,000 $\mu g/m^3$) was identified based on clinical signs of neurotoxicity, decreased bodyweight gain and food consumption, increased water consumption and clinical chemistry parameters. The subchronic NOAEL for the inhalation route is six fold lower than the subchronic NOAEL for the oral route. Therefore, the subchronic inhalation NOAEL of 0.194 mg/kg-day is the critical NOAEL for evaluating seasonal inhalation exposures in people.

Chronic Toxicity and Carcinogenicity Studies in Animals

14. A total of seven chronic studies were available. A two-year dietary study in the rat (Ruckman *et al.*, 1989) and a one-year dietary study in the dog (Brunk, 1989) both identified a NOAEL of 0.6 mg/kg-day. In rats the NOAEL was based on aneurysms, glomerulonephrosis/nephritis, enlarged kidneys, proteinuria and decreased bodyweight gain at 2.9 mg/kg-day. Reduced absolute testes weights

were observed at all dose levels (statistically significant at the two highest dose levels, see Table 2).

Table 2. Mean testes weights from Table 10 of study by Ruckman *et al.* (1989)

Dose level (mg/kg-day)	Mean bodyweight (g)	Mean absolute testes weight (g)
0	748	4.78
0.1	721	4.57
0.3	702	4.17
0.6	690	3.94**
2.9	637	4.04**

William's test; ** $p<0.01$ compared to controls (calculated in study report)

Table 3. Background data (i.e., historical controls) for male rats aged 108-112 weeks from Table 10 of study by Ruckman *et al.* (1989)

	1%	99%
Testes (g)	1.34	6.11
Bodyweights (g)	467	1078

The absolute testes weights fell within the historical control range for this laboratory (Table 3) and there were no histopathological correlates. However, decreased absolute testes weight, relative to the concurrent control, is a well recognized endpoint of male reproductive toxicity and OEHHA would consider them when evaluating the need for an additional safety factor to protect against male reproductive toxicity by endosulfan. Reduced absolute testes weight is also discussed as an indicator of male reproductive toxicity in the Guidelines for Reproductive Toxicity Risk Assessment (US EPA, 1996). In dogs the NOAEL was based on clinical signs of neurotoxicity, premature termination due to animal morbidity and decreased bodyweight gain/food consumption at 2.09 mg/kg-day. No chronic inhalation study was available. Therefore, the subchronic inhalation NOAEL of 0.194 mg/kg-day in the rat was divided by an uncertainty factor of 10 for extrapolation to chronic exposures, yielding an estimated no-effect level (ENEL) of 0.0194 mg/kg-day. Since the critical NOAELs for acute, subchronic and chronic dosing of rats via the oral route were 2.0, 1.18 and 0.6 mg/kg-day, respectively, OEHHA finds that this relatively narrow range (3.3-fold) suggests that the 10-fold uncertainty factor is sufficient for subchronic to chronic extrapolation. This chronic inhalation ENEL is more than 30-fold lower than the chronic dietary NOAELs discussed above that were used in both the Volume I Health Risk Assessment document and by U.S. EPA to evaluate chronic oral exposures.

Two carcinogenicity studies were available that were compliant with the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), one performed in the rat (Ruckman *et al.*, 1989) and one performed in the mouse (Donaubauer, 1988). Both were negative for carcinogenicity. Three older rodent studies (Hazelton for NCI, 1978; Powers *et al.*, 1978 for NCI; WHO, 1984) were also negative for

carcinogenicity, although each had unacceptably high animal mortality and/or other serious methodological problems. A reanalysis of pathology slides from the two National Cancer Institute (NCI) studies of 1978 suggested that both were positive for carcinogenicity (Reuber, 1981, Sci Total Environ **20**: 23-47); however, due to inadequate reporting of how the reanalysis was performed, as well as its unconventional grouping of tumor data, OEHHA finds insufficient justification for disagreeing with the findings of the original pathologists. Based on all the above information, we find that there is insufficient evidence to suggest endosulfan is carcinogenic.

Reproductive Toxicity Studies

15. A number of studies provided data on the reproductive toxicity of endosulfan, and all were conducted by the oral route. A two-generational reproductive toxicity study was performed in the rat (Edwards *et al.*, 1984). The parental NOAEL was 1.1 mg/kg-day in males and 1.3 mg/kg-day in females based on increased kidney and liver weights and decreased bodyweight gain. These were also the reproductive NOAELs, based on a slight decrease in mean litter weight. The ability of males to produce offspring was not affected by endosulfan. In this relatively old study, a number of endpoints of development or function of the reproductive system were not assayed, including sperm counts, crown-rump length, skeletal stains, vaginal opening and preputial separation. As discussed in the RCD, toxicity to the male reproductive system has been observed in a number of LOAEL-only studies from the peer-reviewed literature, albeit at higher exposure levels than those causing subchronic neurotoxicity via inhalation (see Finding 13). One of these studies, by Sinha *et al.* (2001a), is noteworthy for the following reasons. Exposure was gestational at 1.0 mg/kg-day. At 100 days after birth, males exhibited decreased weights of testes, epididymis and seminal vesicles, as well as decreased sperm counts in the cauda epididymis and decreased testicular spermatid head counts. There were no bodyweight effects at the time of sacrifice, changes in dietary intake or clinical signs. There were also no treatment-related changes in litter size or weight. Dividing the study LOAEL by a factor of 10 yields an estimated NOAEL of 0.1 mg/kg-day, which is below the critical subchronic inhalation NOAEL of 0.19 mg/kg-day developed in the RCD. In Dalsenter *et al.* (1999), rats were exposed during gestation and lactation, via the dams. Sperm production was significantly decreased at the lowest dose tested (1.5 mg/kg-day) on post-natal day 65 (puberty) but not on postnatal day 140 (adulthood). There was no maternal toxicity at this dose level as measured by dam bodyweight or litter size. Dividing the study LOAEL by a factor of 10 yields an estimated NOAEL of 0.15 mg/kg-day, which is again below the critical NOAEL developed in the RCD. It should be noted that since these reproductive toxicity studies utilized oral exposures, their NOAELs and LOAELs may be significantly reduced if the route of exposure were changed to inhalation, due to the first pass effect in the liver (see Finding 11).

The RCD also discusses a human epidemiological study of sexual maturation in children living in an agricultural area of India, where endosulfan was the only pesticide reportedly used (Saiyed *et al.*, 2003). Significant delays in the development of reproductive organs were reported in adolescent males, as well as reduced blood levels of testosterone and increased blood levels of luteinizing hormone. Delayed male sexual development (preputial separation) has also been observed in rats treated with endosulfan (Gilmore *et al.*, 2006, see Finding 16).

Developmental Toxicity Studies

16. Two developmental toxicity studies were available in the rat and one in the rabbit. A developmental neurotoxicity study was also available in the rat. Only one of the two rat developmental studies identified NOAELs: a maternal NOAEL of 2.0 mg/kg-day based on clinical signs and decreased bodyweights and a developmental NOAEL of 2.0 mg/kg-day based on reduced fetal weight and length and small or unossified sternbrae (Fung, 1980a). In the rabbit study (Nye, 1981) the maternal NOAEL was 0.7 mg/kg-day based on mortality and clinical signs. No developmental toxicity was observed (developmental NOAEL = 12 mg/kg-day). In the developmental neurotoxicity study in rats (Gilmore *et al.*, 2006), neonates and pups had decreased bodyweights at the lowest dose level tested (3.74 mg/kg-day). This was also the LOAEL for maternal effects, based on lower bodyweights and food consumption. This study detected small delays in preputial separation in males at 10.8 mg/kg-day and vaginal opening in females at 3.74 mg/kg-day. There were no effects on sperm motility, sperm count (normalized to gram of testes or epididymis) or sperm morphology at the highest dose level tested (30 mg/kg-day). Thus, developmental toxicity was not detected at the low inhalation levels that caused subchronic neurotoxicity (see Finding 13). It should be noted that since these studies utilized oral exposures, their NOAELs and LOAELs may be significantly reduced if the route of exposure were changed to inhalation, due to the first pass effect in the liver (see Finding 11).

The RCD also discusses a recent human epidemiological study of autism spectrum disorders (ASD) in children born to mothers who lived in California farm areas where pesticides had been applied during their first eight weeks of pregnancy (Roberts et al., 2007). A significant association was measured between maternal proximity to sites of organochlorine (endosulfan and dicofol) pesticide application and ASD. The authors concluded that the association should be studied further.

Neurotoxicity Studies in Animals

17. A number of neurotoxicity studies were available, primarily in the rat. An acute neurotoxicity study in rats (gavage) showed a greater sensitivity of females compared to males (Bury, 1997). The female NOAEL was 1.5 mg/kg and the male NOAEL was 12.5 mg/kg, both based on mortality and clinical signs. The developmental neurotoxicity study in the rat (dietary) covered the dosing of

females from gestation day six through lactation day 21. The maternal LOAEL and pup developmental LOAEL were both 3.74 mg/kg-day (lowest dose level tested), based on decreased bodyweights. No neurological effects were observed in either the dams or pups (highest dose level tested = 30 mg/kg-day). In a recently published study (Cabaleiro *et al.*, 2008, Effects of *in utero* and lactational exposure to endosulfan in prefrontal cortex of male rats. Toxicol. Lett. 176:58-67), rat offspring were exposed to endosulfan via the dams during the last 13 days of gestation and 21 days of lactation. Male offspring were sacrificed on post-natal days 15, 30 and 60, and the levels of small molecule neurotransmitters were measured in the prefrontal cortex of the brain. The lowest dose level tested of 0.6 mg/kg-day caused significant alterations in the content of a number of transmitting amino acids and amines. In contrast, bodyweights of dams and litter sizes were not significantly affected at this dose level, and bodyweights of the male offspring were not significantly different from controls at four of five time points. Dividing the study LOAEL by a factor of 10 yields an estimated NOAEL of 0.06 mg/kg-day, which is below the critical inhalation NOAEL of 0.194 mg/kg-day developed in the RCD. It should be noted that since these studies utilized oral exposures, their NOAELs and LOAELs may be significantly reduced if the route of exposure were changed to inhalation, due to the first pass effect in the liver (see Finding 11). A study in hens failed to detect any delayed neurotoxicity (Roberts and Phillips, 1983).

Genotoxicity

18. Gene mutation studies were performed with endosulfan in bacteria, yeast, mouse lymphoma cells and *Drosophila* (sex-linked recessive lethals). Both positive and negative results were reported. Chromosome damage was tested *in vivo* and in cultured cells by measuring chromosome aberrations (positive, *in vivo*, germ cell *in vivo* {e.g., Pandey *et al.* 1990, Mutat Res **242**: 1-7}, negative, *in vitro, in vivo*), micronuclei (negative, *in vivo*, positive *in vivo* {e.g., Lajmanovich *et al.*, 2005, Mutat Res **587**: 67-72, Neuparth *et al.*, 2006, Bull Environ Contam Toxicol **76**: 242-8}, positive *in vitro* {e.g., Pistl *et al.*, 2001, Vet Hum Toxicol **43**: 78-82}), sister chromatid exchange (SCE) (positive *in vivo, in vitro* {e.g., Lu *et al.*, 2000, Environ Health Perspect **108**: 559-61}), and dominant lethal induction (positive, negative). Additional studies included unscheduled DNA synthesis in cultured rat hepatocytes (negative), DNA adduct formation in cultured human and rat cells (positive), gene conversion in yeast (positive and negative) and DNA strand breaks (positive, *in vitro* {e.g., Bajpayee *et al.*, 2006, Environ Mol Mutagen **47**: 682-92}, *in vivo* {e.g., Pandey *et al.*, 2006, Ecotoxicol Environ Saf **65**: 56-61}). Thus, while several standard assays were negative, there is evidence that endosulfan is genotoxic.

Calculating Margins of Exposure (MOEs) for Characterizing Human Health Risks

19. OEHHA agrees with the critical NOAELs selected in the RCD for calculating short-term, seasonal, and annual margins of exposure. The critical study for all

three timeframes is the inhalation study of Hollander *et al.* (1984). This was performed with male and female rats, exposed nose-only for 6 hr/day, 5 days/week for 21 days. The LOAEL was 0.387 mg/kg-day based on clinical signs, decreased bodyweight gain and food consumption, increased water consumption and clinical chemistry parameters. The NOAEL was 0.194 mg/kg-day. This NOAEL was used directly for short-term and seasonal MOE calculations. For calculating annual MOEs, an estimated no-effect level, or ENEL, was derived by dividing the NOAEL from the 21-day inhalation study by an uncertainty factor of 10 for extrapolation from subchronic (seasonal) to chronic (annual) exposures. As discussed in more detail in Finding 14, OEHHA would use the same approach for extrapolating to chronic (annual) exposures.

20. In the Volume I Health Risk Assessment document, MOEs were calculated by dividing the appropriate NOAEL (or ENEL) by the exposure. Short-term, seasonal and annual inhalation MOEs were calculated for infants and adults exposed as bystanders (Table 4). When using NOAELs from animal studies, DPR considers MOEs of greater than 100 to be health protective, regardless of the route of exposure. Specifically for inhalation exposures to the general public, MOEs of less than 1000 indicate that a chemical should be identified as a TAC.

Aggregate MOEs, based on inhalation and dietary exposures, are also shown in Table 4. The dietary components are based on the 95th percentile of daily dietary intake of endosulfan by nursing females 13+years old (for short-term aggregate MOEs) or the mean daily dietary intake of endosulfan by nursing females 13+ years old (for seasonal and annual aggregate MOEs).

Table 4. Margins of Exposure (MOEs) in the Volume I Health Risk Assessment document for Short-Term, Seasonal and Annual Exposures to Endosulfan via Bystander Inhalation Only, or via Bystander Inhalation + Dietary (i.e., Aggregate)

	Infants	Adults
Short-term Inhalation MOEs	121	255
Seasonal Inhalation MOEs	346	719
Annual Inhalation MOEs	413	882
Short-term Aggregate MOEs	78	146
Seasonal Aggregate MOEs	296	595
Annual Aggregate MOEs	343	702

21. Inhalation MOEs (Table 4) ranged from 121 to 882. Adding in dietary exposure gave lower MOEs, ranging from 78 to 702. Infants had the lowest short-term aggregate MOE of 78. For this group, 67 percent of the exposure to endosulfan was through the diet and 33 percent was through the air. We note that all MOEs, both inhalation-only and aggregate, were below 1000, making endosulfan a potential TAC.

22. Reference concentrations are calculated in the Volume I Health Risk Assessment document for acute, subchronic and chronic exposures to endosulfan based on the

NOAEL of 0.194 mg/kg-day from the subchronic rat inhalation study (Table 5). For acute and subchronic RfCs, an uncertainty factor of ten was applied for animal to human extrapolation and ten for human variability. For chronic RfC calculation, an additional uncertainty factor of ten was applied to extrapolate from subchronic to chronic exposure. As discussed in detail in Finding 23 below, OEHHA would add an uncertainty factor of no more than three to the infant calculations, yielding acute, subchronic and chronic RfCs that are three fold lower than those calculated in the RCD (Table 5).

Table 5. Reference Concentrations (RfCs) calculated in the Volume I Health Risk Assessment document or by OEHHA for Acute, Subchronic and Chronic Exposures to Endosulfan

	Infants	Adults
Calculated in Volume I Health Risk Assessment		
Acute	3.3 $\mu g/m^3$	6.9 $\mu g/m^3$
Subchronic	3.3 $\mu g/m^3$	6.9 $\mu g/m^3$
Chronic	0.33 $\mu g/m^3$	0.69 $\mu g/m^3$
Calculated by OEHHA adding an uncertainty factor of 3 for infants (see Finding 23)		
Acute	1.1 $\mu g/m^3$	6.9 $\mu g/m^3$
Subchronic	1.1 $\mu g/m^3$	6.9 $\mu g/m^3$
Chronic	0.11 $\mu g/m^3$	0.69 $\mu g/m^3$

The RfC values in the RCD and reproduced in Table 5 can be compared to the concentrations calculated for infants and adults exposed to endosulfan as bystanders (Table 6). All six fractional RfC values are greater than ten percent, indicating that endosulfan should be identified as a TAC. Note that if the infant RfCs were reduced by a factor of three as proposed by OEHHA, the percent RfC values for infants would be even larger.

Table 6. Percent Reference Concentrations for Bystander Inhalation Exposures Estimated in the Volume I Health Risk Assessment document

	Endosulfan Air Concentration as a Percentage of RfC*	
	Infants	Adults
Acute (short-term)	82%	39%
Subchronic (seasonal)	29%	14%
Chronic (annual)	24%	11%

*Endosulfan air concentration as a percentage of RfC was calculated by dividing the exposure rate for each exposure scenario (Tables 1 of these findings) by the breathing rate, and expressing each of those values as a percentage of the corresponding RfC

Additional Findings

23. The subchronic inhalation study by Hollander *et al.* (1984) was performed with four to six week-old rats. As such, OEHHA considers its LOAEL and NOAEL appropriate for adult risk assessment, and would apply an uncertainty factor for interspecies, intraspecies and subchronic to chronic extrapolation. This is also the

approach followed in the Volume I Health Risk Assessment document for adults. However, in performing inhalation risk assessment for the effects that may only result from exposures to rats developing *in utero* or postnatally, the document must rely on studies where dosing was via the diet or gavage, with the times of exposure varying from gestational (developmental toxicity), to gestational/lactational (developmental neurotoxicity) to multigenerational (reproductive toxicity). For the following reasons, OEHHA finds significant uncertainty associated with using these studies to predict how young rats and rats *in utero* would respond to endosulfan via inhalation:

- There are a number of studies where endosulfan in the range of 0.6 to 1.5 mg/kg-day produced developmental neurotoxicity (Cabaleiro *et al.*, 2008; see Finding 17) and male reproductive toxicity in rats, including reduced testes weight and/or function (Ruckman *et al.*, 1989; Dalsenter *et al.*, 1999, Sinha *et al.*, 2001a). The studies of Dalsenter *et al.* (1999), Sinha *et al.* (2001a) and Cabaleiro *et al.* (2008) were LOAEL-only studies, which if extrapolated to a NOAEL by application of an uncertainty factor of 10, would be as much as 2 to 3-fold below the critical subchronic inhalation NOAEL of 0.194 developed in the RCD. In addition, since these were oral exposures, either directly to young rats or indirectly via dams, and a significant first pass effect in the liver was operating (see Finding 11), the levels of endosulfan reaching the general circulation were likely lower than if exposure had been via inhalation. Therefore, uncertainty remains as to whether these effects would be absent after inhalation exposures to young rats or rats *in utero* at the critical subchronic inhalation NOAEL of 0.194 mg/kg-day.

- There has been inadequate testing during the post-lactational period of rat development, starting at approximately three weeks after birth. This is a period normally covered by a reproductive toxicity study. Such a study is available for endosulfan (Edwards *et al.*, 1984); however, a number of developmental endpoints required by today's guidelines were not required at that time, including sperm counts, spermatid number, sperm motility, sperm morphology, crown-rump length, skeletal stains, vaginal opening and preputial separation. The developmental neurotoxicity study by Gilmore *et al.* (2006) attempted to address some of these shortcomings. Reduced pup weights and delayed preputial separation were observed, indicating developmental toxicity. Insufficient reporting and analysis of testicular endpoints (testes weights and sperm analysis) measured 55 days after dosing had ceased, limits the usefulness of this dataset. Importantly, Gilmore *et al.*'s (2006) failure to extend dosing to the post-lactational period of development precludes its use as a substitute for a guideline reproductive toxicity study, in which the four sperm parameters listed above are measured in animals exposed continuously from conception through puberty. Given that humans, unlike rats, do not produce sperm in large excess of what is required for normal fertility (Amann, 1986, Detection of alterations in testicular and epididymal function in laboratory animals. Environ Health Perspect. 70:149-158), the failure of Gilmore *et al.* (2006) to measure these sperm parameters in animals exposed during the post-lactational period of development

cannot be corrected by measurements of offspring production by males in Edwards *et al.* (1984).

- There is some evidence that young rats are more sensitive to endosulfan than adults. Table 7 shows these comparisons. For Seth *et al.* (1986), dosing was by intraperitoneal injection (ip). For the Sinha *et al.* studies (1995 and 1997), dosing was by gavage. In both the Seth *et al.* (1986) study and in the two Sinha *et al.* (1995, 1997) studies, the pups exhibited these endpoints of possible developmental neurotoxicity and male reproductive toxicity at lower dose levels than the older animals. The difference was at least 3-fold in Seth *et al.* (1986) and at most 2-fold in Sinha *et al.* (1995, 1997). The bases for these age effects are unknown. An important consideration is whether this pattern of age-related sensitivity would be repeated for these or other endpoints under the conditions of the critical subchronic inhalation study. In the absence of data, OEHHA would add an uncertainty factor to help protect against this possibility. It should be noted that since these studies utilized oral or ip dosing, their NOAELs and LOAELs may be significantly reduced if the route of exposure were changed to inhalation, due to the first pass effect in the liver (see Finding 11).

Table 7 comparing rat pup and adult sensitivities to endosulfan: effects on brain chemistry, behavior and sperm parameters

Study	Age	Dosing	Dose level producing effects	Endpoints tested and effects observed
Seth *et al.*, 1986	pups at birth	ip over 5 weeks (25 treatments)	1 mg/kg-day	Increased binding in brain of serotonin and benzodiazepine, decreased dopamine binding, increased fighting, all at 8 days after cessation of dosing
	adults (8 weeks old)	ip over 30 days (30 treatments)	No effect at highest dose tested (3 mg/kg-day)	Serotonin binding in brain, fighting behavior, all at 8 days after cessation of dosing
Sinha *et al.*, 1997	3 week old pups	daily gavage for 10 weeks	2.5 mg/kg-day	Decreased spermatid count and sperm production rate, increased sperm abnormalities
Sinha *et al.*, 1995	3 month old adults	daily gavage for 10 weeks	5 mg/kg-day, no effect at 2.5 mg/kg-day	Decreased spermatid count and sperm production rate, increased sperm abnormalities

In Table 7, the effects of exposure to endosulfan occurred at a two to three-fold lower dose level in the young rats compared to the adults. Therefore, to protect against this age-related sensitivity, in consideration of the LOAEL-only studies

(gestational and lactational dosing) and their estimated NOAELs that are two to three-fold lower than the critical inhalation NOAEL developed in the RCD (see first bullet above), as well as to account for the pharmacokinetic and testing uncertainties discussed above, OEHHA would apply an additional uncertainty factor of no more than three in calculating the infant RfCs shown in Tables 5 and 6.

24. In animal tests, technical grade endosulfan caused dermal irritation but was not irritating to the eye. Endosulfan formulated products caused both dermal and ocular irritation. In the guinea pig dermal sensitization test, two endosulfan formulations were negative and one was a moderate dermal sensitizer. Thus, there is a potential risk of dermal sensitization in humans exposed to endosulfan.

25. One study from the published literature found no evidence for cumulative toxicity involving endosulfan and other organochlorine compounds.